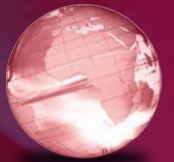


GLOBAL
EDITION



Biology

A Global Approach

ELEVENTH EDITION

Campbell
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GLOBAL EDITION

BIOLOGY

A Global Approach

ELEVENTH EDITION



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About the Authors



Neil A. Campbell (1946–2004) earned his M.A. from the University of California, Los Angeles, and his Ph.D. from the University of California, Riverside. His research focused on desert and coastal plants. Neil's 30 years of teaching included introductory biology courses at Cornell University, Pomona College, and San Bernardino Valley College, where he received the college's first Outstanding Professor Award in 1986. For many years he was also a visiting scholar at UC Riverside. Neil was the founding author of *BIOLOGY*.



Lisa A. Urry is Professor of Biology and Chair of the Biology Department at Mills College. After earning a B.A. at Tufts University, she completed her Ph.D. at the Massachusetts Institute of Technology (MIT). Lisa has conducted research on gene expression during embryonic and larval development in sea urchins. Deeply committed to promoting opportunities in science for women and underrepresented minorities, she has taught courses ranging from introductory and developmental biology to a nonmajors course called Evolution for Future Presidents. Lisa is a coauthor of *Campbell Biology in Focus*.



Michael L. Cain is an ecologist and evolutionary biologist who is now writing full-time. Michael earned an A.B. from Bowdoin College, an M.Sc. from Brown University, and a Ph.D. from Cornell University. As a faculty member at New Mexico State University, he taught introductory biology, ecology, evolution, botany, and conservation biology. Michael is the author of dozens of scientific papers on topics that include foraging behavior in insects and plants, long-distance seed dispersal, and speciation in crickets. He is a coauthor of *Campbell Biology in Focus* and of an ecology textbook.



Steven A. Wasserman is Professor of Biology at the University of California, San Diego (UCSD). He earned an A.B. from Harvard University and a Ph.D. from MIT. Working on the fruit fly *Drosophila*, Steve has done research on developmental biology, reproduction, and immunity. Having taught genetics, development, and physiology to undergraduate, graduate, and medical students, he now focuses on introductory biology, for which he has been honored with UCSD's Distinguished Teaching Award. He is a coauthor of *Campbell Biology in Focus*.



Peter V. Minorsky is Professor of Biology at Mercy College in New York, where he teaches introductory biology, ecology, and botany. He received his A.B. from Vassar College and his Ph.D. from Cornell University. Peter taught at Kenyon College, Union College, Western Connecticut State University, and Vassar College; he is also the science writer for the journal *Plant Physiology*. His research interests concern how plants sense environmental change. Peter received the 2008 Award for Teaching Excellence at Mercy College and is a coauthor of *Campbell Biology in Focus*.



Jane B. Reece, the head of the author team for Editions 8–10 of *BIOLOGY*, was Neil Campbell's longtime collaborator. Jane taught biology at Middlesex County College and Queensborough Community College. She holds an A.B. from Harvard University, an M.S. from Rutgers University, and a Ph.D. from the University of California, Berkeley. Jane's research as a doctoral student at UC Berkeley and postdoctoral fellow at Stanford University focused on genetic recombination in bacteria. Besides her work on *BIOLOGY*, Jane has been a coauthor on all the *Campbell* texts.

Preface

We are honored to present *BIOLOGY: A Global Approach*, which has been adapted from *Campbell BIOLOGY*, Eleventh Edition, for a global audience. For the last three decades, *BIOLOGY* has been the leading college text in the biological sciences. It has been translated into 19 languages and has provided millions of students with a solid foundation in college-level biology. This success is a testament not only to Neil Campbell's original vision but also to the dedication of hundreds of reviewers (listed on pages 28–32), who, together with editors, artists, and contributors, have shaped and inspired this work.

Our goals for the Eleventh Edition include:

- **increasing visual literacy** through new figures, questions, and exercises that build students' skills in understanding and creating visual representations of biological structures and processes
- asking students to **practice scientific skills** by applying scientific skills to real-world problems
- **supporting instructors** by providing teaching modules with tools and materials for introducing, teaching, and assessing important and often challenging topics
- **integrating text and media** to engage, guide, and inform students in an active process of inquiry and learning

Our starting point, as always, is our commitment to crafting text and visuals that are accurate, are current, and reflect our passion for teaching biology.

New to This Edition

Here we provide an overview of the new features that we have developed for the Eleventh Edition; we invite you to explore pages 9–26 for more information and examples.

- **Visualizing Figures** and **Visual Skills Questions** give students practice in interpreting and creating visual representations in biology. Assignable questions are also available in **MasteringBiology** to give students practice with the visual skills addressed in the figures.
- **Problem-Solving Exercises** challenge students to apply scientific skills and interpret data in solving real-world problems. These exercises are designed to engage students through compelling case studies and provide practice with data analysis skills. Problem-Solving Exercises have assignable versions in **MasteringBiology**. Some also have more extensive “Solve It” investigations to further explore a given topic.



- **Ready-to-Go Teaching Modules** on key topics provide instructors with assignments to use before and after class, as well as in-class activities that use clickers or Learning Catalytics™ for assessment.
- **Integrated text and media:** Media references in the printed book direct students to the wealth of online self-study resources available to them in the **Study Area** section of **MasteringBiology**. The new online learning tools include:
 - **Get Ready for This Chapter** questions provide a quick check of student understanding of the background information needed to learn a new chapter's content, with feedback to bolster their preparation.
 - **Figure Walkthroughs** guide students through key figures with narrated explanations, figure markups, and questions that reinforce important points. Additional questions can be assigned in **MasteringBiology**.
 - More than **450 animations and videos** bring biology to life. These include resources from **HHMI BioInteractive** that engage students in topics from the discovery of the double helix to evolution.
 - QR codes and URLs within the Chapter Review provide easy access to **Vocabulary Self-Quizzes** for each chapter that can be used on smartphones, tablets, and computers.
 - **Interviews** from the First Edition through the Eleventh Edition of *BIOLOGY* are referenced in the chapter where they are most relevant. The interviews show students the human side of science by featuring diverse scientists talking about how they became interested in what they study, how they began, and what inspires them.
- The impact of **climate change** at all levels of the biological hierarchy is explored throughout the text, starting with a new figure (Figure 1.12) and discussion in Chapter 1 and concluding with a new Make Connections Figure (Figure 56.30) and expanded coverage on causes and effects of climate change in Chapter 56.
- As in each new edition of *BIOLOGY*, the Eleventh Edition incorporates **new content** and **pedagogical improvements**. These are summarized on pp. 6–8, following this Preface. Content updates reflect rapid, ongoing changes in technology and knowledge in the fields of genomics, gene editing technology (CRISPR), evolutionary biology, microbiology, and more. In addition, significant revisions to Unit 8, The Ecology of Life, improve the conceptual framework for core ecological topics (such

as population growth, species interactions, and community dynamics) and more deeply integrate evolutionary principles.

Our Hallmark Features

Teachers of general biology face a daunting challenge: to help students acquire a conceptual framework for organizing an ever-expanding amount of information. The hallmark features of *BIOLOGY* provide such a framework, while promoting a deeper understanding of biology and the process of science. As such, they are well aligned with the core competencies outlined by the 2009 **Vision and Change** national conference, organized by the American Association for the Advancement of Science, where more than 500 biologists met to discuss the needs of undergraduate biology. Furthermore, the core concepts defined by Vision and Change have close parallels in the unifying themes that are introduced in Chapter 1 and integrated throughout the book. Chief among the themes of both Vision and Change and *BIOLOGY* is **evolution**. Each chapter of this text includes at least one Evolution section that explicitly focuses on evolutionary aspects of the chapter material, and each chapter ends with an Evolution Connection Question and a Write About a Theme Question.

To help students distinguish the “forest from the trees,” each chapter is organized around a framework of three to seven carefully chosen **Key Concepts**. The text, Concept Check Questions, Summary of Key Concepts, and MasteringBiology resources all reinforce these main ideas and essential facts.

Because text and illustrations are equally important for learning biology, **integration of text and figures** has been a hallmark of this text since the First Edition. In addition to the new Visualizing Figures, our popular Exploring Figures and Make Connections Figures epitomize this approach. Each Exploring Figure is a learning unit of core content that brings together related illustrations and text. Make Connections Figures reinforce fundamental conceptual connections throughout biology, helping students overcome tendencies to compartmentalize information. The Eleventh Edition features two new Make Connections Figures. There are also Guided Tour Figures that walk students through complex figures as an instructor would.

To encourage **active reading** of the text, *BIOLOGY* includes numerous opportunities for students to stop and think about what they are reading, often by putting pencil to paper to draw a sketch, annotate a figure, or graph data. Active reading questions include Visual Skills Questions, Draw It Questions, Make Connections Questions, What If? Questions, Figure Legend Questions, Summary Questions, Synthesize Your Knowledge Questions, and Interpret the Data Questions. Answering these questions requires students to write or draw as well as think and thus helps develop the core competency of communicating science.

Finally, *BIOLOGY* has always featured **scientific inquiry**, an essential component of any biology course. Complementary stories of scientific discovery in the text narrative and the

unit-opening interviews, our standard-setting Inquiry Figures deepen the ability of students to understand how we know what we know. Scientific Inquiry Questions give students opportunities to practice scientific thinking, along with the Problem-Solving Exercises, Scientific Skills Exercises, and Interpret the Data Questions. Together, these activities provide students practice in both applying the process of science and using quantitative reasoning, addressing additional core competencies outlined in Vision and Change.

MasteringBiology®

MasteringBiology, the most widely used online assessment and tutorial program for biology, provides an extensive library of homework assignments that are graded automatically. In addition to the **new Get Ready for This Chapter Questions, Figure Walkthroughs, Problem-Solving Exercises, and Visualizing Figures**, MasteringBiology offers Dynamic Study Modules, Adaptive Follow-Up Assignments, Scientific Skills Exercises, Interpret the Data Questions, Solve It Tutorials, HHMI Bio-Interactive Short Films, BioFlix® Tutorials with 3-D Animations, Experimental Inquiry Tutorials, Interpreting Data Tutorials, BLAST Tutorials, Make Connections Tutorials, Video Field Trips, Video Tutor Sessions, Get Ready for Biology, Activities, Reading Quiz Questions, Student Misconception Questions, 4,500 Test Bank Questions, and MasteringBiology Virtual Labs. MasteringBiology also includes the *BIOLOGY* eText, Study Area, Instructor Resources, and Ready-to-Go Teaching Modules. See pages 9–23 and www.masteringbiology.com for more details.

Our Partnership with Instructors and Students

A core value underlying our work is our belief in the importance of a partnership with instructors and students. One primary way of serving instructors and students, of course, is providing a text that teaches biology well. In addition, Pearson offers a rich variety of instructor and student resources, in both print and electronic form (see pp. 9–23). In our continuing efforts to improve the book and its supplements, we benefit tremendously from instructor and student feedback, not only in formal reviews from hundreds of scientists, but also via e-mail and other avenues of informal communication.

The real test of any textbook is how well it helps instructors teach and students learn. We welcome comments from both students and instructors. Please address your suggestions to:

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Highlights of New Content

This section highlights selected new content and pedagogical changes in *BIOLOGY*, Eleventh Edition.

CHAPTER 1 Biology and Its Themes

Chapter 1 opens with a new introduction to a case study on the evolution of coloration in mice. New text and a new photo (Figure 1.12) relate climate change to species survival.

UNIT 1 THE ROLE OF CHEMISTRY IN BIOLOGY

In Unit 1, new content engages students in learning this foundational material. The opening of Chapter 3 and new **Figure 3.7** show organisms affected by **loss of Arctic sea ice**. Chapter 5 has updates on lactose intolerance, *trans* fats, the effects of diet on blood cholesterol, protein sequences and structures, and intrinsically disordered proteins.

New Visualizing Figure 5.16 helps students understand various ways proteins are depicted. A new Problem-Solving Exercise engages students by having them compare DNA sequences in a case of possible fish fraud. Chapter 6 includes a beautiful new photo of a geyser with thermophilic bacteria in Figure 6.17, bringing to life the graphs of optimal temperatures for enzyme function.

▼ **Figure 3.7 Effects of climate change on the Arctic.**



UNIT 2 CELL BIOLOGY

Our main goal for this unit was to make the material more accessible and inviting to students. New Visualizing Figure 7.32 shows the profusion of molecules and structures in a cell, all drawn to scale. In Chapter 8, a new figure illustrates levels of LDL receptors in people with and without familial hypercholesterolemia. Chapter 9 includes a new Problem-Solving Exercise that guides students through assessing possible new treatments for bacterial infections by blocking quorum sensing. Chapter 11 discusses current research trying to genetically modify rice (a C_3 crop) so that it is capable of carrying out C_4 photosynthesis to increase yields. In Chapter 12, the mechanism of chromosome movement in bacteria has been updated and more cell cycle control checkpoints have been added, including one proposed by researchers in 2014.

UNIT 3 THE GENETIC BASIS OF LIFE

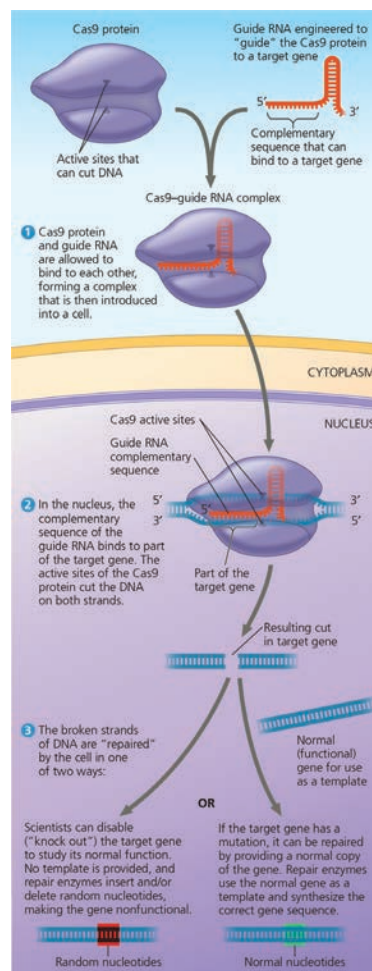
In Chapters 13–17, we have incorporated changes that help students grasp the more abstract concepts of genetics and their chromosomal and molecular underpinnings. For example, a new Visual Skills Question with Figure 13.6 asks students to identify where in the three life cycles haploid cells undergo mitosis, and

what type of cells are formed. Chapter 14 includes new information from a 2014 genomic study on the number of genes and genetic variants contributing to height. Figure 14.15b now uses “inability to taste PTC” rather than “attached earlobe.” Chapters 14 and 15 are more inclusive, clarifying the meaning of the term “normal” in genetics and explaining that sex is no longer thought to be simply binary. Other updates in Chapter 15 include new research in sex determination and a technique being developed to avoid passing on mitochondrial diseases. New Visualizing Figure 16.7 shows students various ways that DNA is illustrated. Chapter 17 has a new opening photo and story about albino donkeys to pique student interest in gene expression. To help students understand the Beadle and Tatum experiment, new Figure 17.2 explains how they obtained nutritional mutants. A new Problem-Solving Exercise asks students to identify mutations in the insulin gene and predict their effect on the protein.

Chapters 18–20 are extensively updated, driven by exciting new discoveries based on DNA sequencing and gene-editing technology. Chapter 18 has updates on histone modifications, nuclear location and the persistence of transcription factories, chromatin remodeling by ncRNAs, long noncoding RNAs (lncRNAs), the role of master regulatory genes in modifying chromatin structure, and the possible role of *p53* in the low incidence of cancer in elephants. Make Connections Figure 18.27, “Genomics, Cell Signaling, and Cancer,” has been expanded to include more information on cell signaling. Chapter 19 has a new photo of next-generation DNA sequencing machines (Figure 19.2) and a new illustration of the widely used technique of RNA sequencing (Figure 19.13). A new section titled Editing Genes and Genomes has been added describing the **CRISPR-Cas9 system** (Figure 19.14)

that has been developed to edit genes in living cells. Information has also been added later in the chapter on use of the CRISPR-Cas9 system, including a study in which a genetic mutation for the disease tyrosinemia was corrected in mice. Finally, the discussion of ethical considerations has been updated to include a recent report of scientists using the CRISPR-Cas9 system to edit a gene in human embryos, along with a discussion of the ethical questions raised by such experiments, such as its usage in the gene drive approach to combat carrying of diseases

▼ **Figure 19.14 Gene editing using the CRISPR-Cas9 system.**

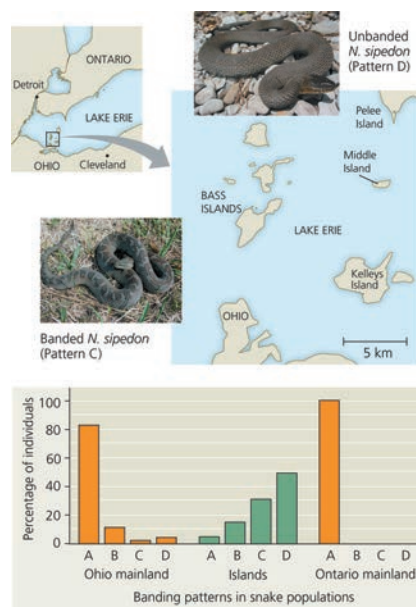


by mosquitoes. In Chapter 20, in addition to the usual updates of sequence-related data (speed of sequencing, number of species' genomes sequenced, etc.), there are several research updates, including some early results from the new Roadmap Epigenomics Project and results from a 2015 study focusing on 414 important yeast genes.

UNIT 4 EVOLUTION

A major goal for this revision was to strengthen how we help students understand and interpret visual representations of evolutionary data and concepts. Toward this end, we have added a new figure (Figure 25.8), “Visualizing the Scale of Geologic Time,” and a new figure (Figure 23.12) on **gene flow**. Another new figure (Figure 22.5, “Visualizing Phylogenetic Relationships”) introduces the visual conventions used in phylogenetic trees and helps students understand what such trees do and don't convey. Several figures have been revised to improve the presentation of data, including Figure 24.6 (on reproductive isolation in mosquitofish), Figure 24.10 (on allopolyploid speciation), and Figure 25.25 (on the origin of the insect body plan). The unit also features new material that connects evolutionary concepts and societal problems. Examples include text in Chapter 21 on the 2015 discovery of teixobactin, an antibiotic that is effective against some hard-to-treat pathogens, a new discussion in Chapter 24 on the impact of climate change on hybrid zones, and a new Problem-Solving Exercise in Chapter 24 on how hybridization may have led to the spread of insecticide resistance genes in mosquitoes that transmit malaria. The unit also includes new chapter-opening stories in Chapter 21 (on a moth whose features illustrate the concepts of unity, diversity, and adaptation) and Chapter 25 (on the discovery of whale bones in the Sahara Desert). Additional changes include new text in Concept 21.3 emphasizing how populations can evolve over short periods of time, new text and a new figure (Figure 22.22) on horizontal gene transfer from prokaryotes to eukaryotes, a new table (Table 23.1) highlighting the five conditions required for a population to be in Hardy-Weinberg equilibrium, and new material in Concept 25.1 describing how researchers recently succeeded for the first time in constructing a “protocell” in which replication of a template strand of RNA could occur.

▼ **Figure 23.12 Gene flow and local adaptation in the Lake Erie water snake (*Nerodia sipedon*).**



UNIT 5 THE DIVERSITY OF LIFE

Chapter 26, the first chapter of this unit, features a new section that covers bacterial defenses against bacteriophages and describes the CRISPR-Cas9 system (Figure 26.7); updates include the Ebola,

Chikungunya, and Zika viruses (Figure 26.10) and discovery of the largest virus known to date. A discussion has been added of mosquito transmission of diseases and concerns about the effects of global climate change on disease transmission. Students are provided many opportunities to practice their visual skills, with many new Visual Skills Questions on topics ranging from interpreting phylogenetic trees to predicting which regions of a bacterial flagellum are hydrophobic. The unit also contains new content on tree thinking, emphasizing such key points as how sister groups provide a clear way to describe evolutionary relationships and how trees do not show a “direction” in evolution. Other major content changes include new text in Concepts 27.4, and 28.1 on the 2015 discovery of the Lokiarchaeota, a group of archaea that may represent the sister group of the eukaryotes, new text in Concept 27.6 describing the CRISPR-Cas9 system and a new figure (Figure 27.21) that illustrates one example of how CRISPR-Cas 9 technology has opened new avenues of research on HIV, and new material in Concept 29.3 describing how early forests contributed to global climate change (in this case, global cooling). A new Problem-Solving Exercise in Chapter 34 engages students in interpreting data from a study investigating whether frogs can acquire resistance to a fungal pathogen through controlled exposure to it. Other updates include the revision of many phylogenies to reflect recent phylogenomic data, new chapter-opening stories in Chapter 31 (on how mycorrhizae link trees of different species) and Chapter 33 (on the “blue dragon,” a mollusc that preys on the highly toxic Portuguese man-of-war), new text and a new figure (Figure 34.37) on the adaptations of the kangaroo rat to its arid environment, and new material in Concept 34.7, including a new figure (Figure 34.52) describing fossil and DNA evidence indicating that humans and Neanderthals interbred, producing viable offspring. The discussion of **human evolution** also includes new text and a new figure (Figure 34.53) on *Homo naledi*, the most recently discovered member of the human evolutionary lineage.

▼ **Figure 34.53 Fossils of hand and foot bones of *Homo naledi*.**



UNIT 6 PLANTS: STRUCTURE AND FUNCTION

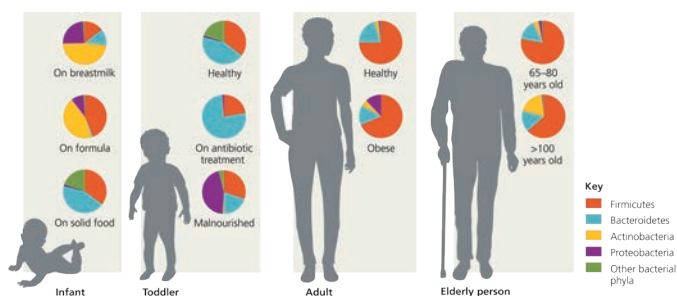
A major aim in revising Chapter 35 was to help students better understand how primary and secondary growth are related. New Visualizing Figure 35.11 enables students to picture growth at the cellular level. Also, the terms *protoderm*, *procambium*, and *ground meristem* have been introduced to underscore the transition of meristematic to mature tissues. A new flowchart (Figure 35.24) summarizes growth in a woody shoot. New text and a figure (Figure 35.26) focus on genome analysis of *Arabidopsis* ecotypes, relating plant morphology to ecology and evolution. In Chapter 36, new Figure 36.8 illustrates the fine branching of leaf veins, and information on phloem-xylem water transfer has been updated. New Make Connections Figure 37.10 highlights mutualism across kingdoms and domains. Figure 37.13 and the related text include new findings on how some soil nitrogen derives from weathering of rocks. New Figure 38.3 clarifies how the terms *carpel* and *pistil* are related. The text on flower structure and the angiosperm

life cycle figure identify carpels as megasporophylls and stamens as microsporophylls, correlating with the plant evolution discussion in Unit 5. In Concept 38.3, the current problem of glyphosate-resistant crops is discussed in detail. A revised Figure 39.7 helps students visualize how cells elongate. Figure 39.8 now addresses apical dominance in a Guided Tour format. Information about the role of sugars in controlling apical dominance has been added. In Concept 39.4, a new Problem-Solving Exercise highlights how global climate change affects crop productivity. Figure 39.26 on defense responses against pathogens has been simplified and improved.

UNIT 7 ANIMALS: STRUCTURE AND FUNCTION

A major goal of the Unit 7 revision was to transform how students interact with and learn from representations of anatomy and physiology. For example, gastrulation is now introduced with a Visualizing Figure (Figure 46.8) that provides a clear and carefully paced introduction to three-dimensional processes that may be difficult for students to grasp. In addition, a number of the new and revised figures help students explore spatial relationships in anatomical contexts, such as the interplay of lymphatic and cardiovascular circulation (Figure 43.15) and the relationship of the limbic system to overall brain structure (Figure 49.14). A new Problem-Solving Exercise in Chapter 41 taps into student interest in medical mysteries through a case study that explores the science behind laboratory testing and diagnosis. Content updates help students appreciate the continued evolution of our understanding of even familiar phenomena, such as the sensation of thirst (Concept 44.4) and the locomotion of kangaroos and jellies (Concept 50.6). Furthermore, new text and figures introduce students to cutting-edge technology relating to such topics as RNA-based antiviral defense in invertebrates (Figure 47.4) and rapid, comprehensive characterization of viral exposure (Figure 47.24), as well as recent discoveries regarding brown fat in adult humans (Figure 40.16), the **microbiome** (Figure 42.17), parthenogenesis (Concept 45.1), and magnetoreception (Concept 50.1). As always, there is fine-tuning of pedagogy, as in discussions of the complementary roles of inactivation and voltage gating of ion channels during action potential formation (Concept 48.3).

▼ **Figure 42.17** Variation in human gut microbiome at different life stages.



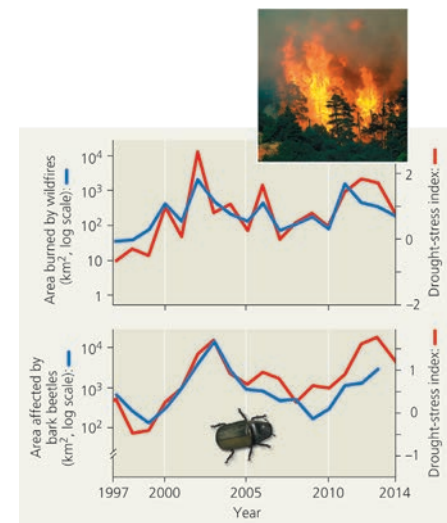
UNIT 8 THE ECOLOGY OF LIFE

The Ecology Unit has been extensively revised for the Eleventh Edition. We have reorganized and improved the conceptual framework with which students are introduced to the

following core ecological topics: life tables, per capita population growth, intrinsic rate of increase (r), exponential population growth, logistic population growth, density dependence, species interactions (in particular, parasitism, commensalism, and mutualism), and MacArthur and Wilson's island biogeography model. The revision also includes a deeper integration of evolutionary principles, including a new Key Concept (51.5) and two new figures (Figures 51.22 and 51.23) on the reciprocal effects of ecology and evolution, new material in Concept 51.4 on how the geographic distributions of species are shaped by a combination of evolutionary history and ecological factors, and five new Make Connections Questions that ask students to examine how ecological and evolutionary mechanisms interact. In keeping with our goal of expanding and strengthening our coverage of climate change, we have added a new discussion and a new figure (Figure 51.20) on how climate change has affected the distribution of a keystone species, a new section of text in Concept 55.2 on how climate change affects NPP, a new figure (Figure 55.8) on how **climate change** has caused an increase in wildfires and insect outbreaks, a new Problem-Solving Exercise in Chapter 55 that explores how insect outbreaks induced by climate change

can cause an ecosystem to switch from a carbon sink to a carbon source, a new figure (Figure 56.29) on the greenhouse effect, new text in Concept 56.4 on biological effects of climate change, and a new Make Connections Figure (Figure 56.30) on how climate change affects all levels of biological organization. Additional updates include a new figure (Figure 53.25) on per capita ecological footprints, a new

▼ **Figure 55.8** Climate change, wildfires, and insect outbreaks.



chapter-opening story in Chapter 54 on a seemingly unlikely mutualism between a shrimp and a much larger predatory fish, new text in Concept 54.1 emphasizing that each partner in a mutualism experiences both benefits and costs, new text in Concept 54.1 describing how the outcome of an ecological interaction can change over time, two new figures (Figures 54.29 and 54.30) on the island equilibrium model, a new figure (Figure 54.31) documenting two shrew species as unexpected hosts of the Lyme disease, new text in Concept 56.1 comparing extinction rates today with those typically seen in the fossil record, and a new discussion and figure (Figure 56.22) on the restoration of a degraded urban stream.

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Ready-to-Go Teaching Modules

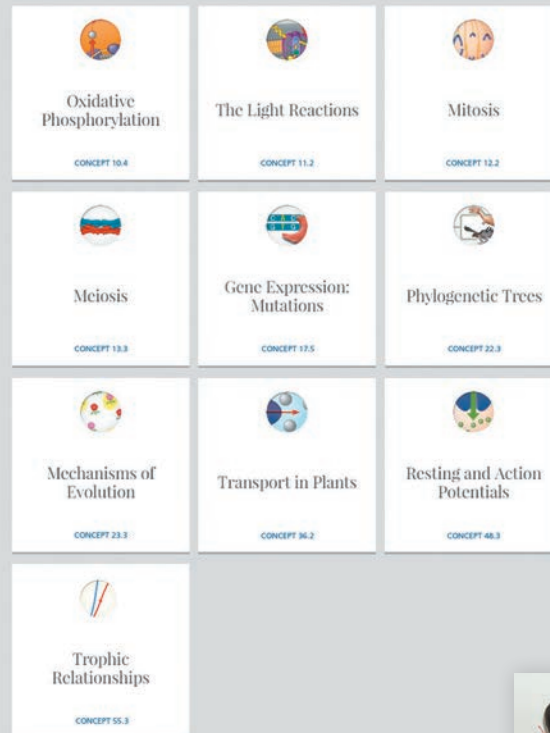


Ready-to-Go Teaching Modules provide instructors with easy-to-use teaching tools for the toughest topics in General Biology.

Assign ready-made activities and assignments for before, during, and after class.

Incorporate active learning with class-tested resources from biology instructors.

Take full advantage of MasteringBiology and Learning Catalytics™, the powerful "bring your own device" student assessment system.

The **Ready-to-Go Teaching Modules** incorporate the best that the text, MasteringBiology, and Learning Catalytics have to offer, along with new ideas for in-class activities. The modules can be accessed through the Instructor Resources area of MasteringBiology.

Instructors can easily incorporate **active learning** into their courses using suggested activity ideas and questions. Videos demonstrate how the activities can be used in class.



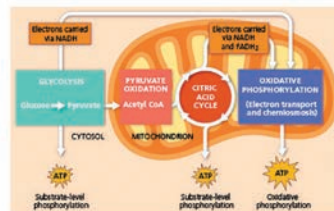
Learning Catalytics™ allows students to use their smartphone, tablet, or laptop to respond to questions in class. Visit learningcatalytics.com.

The following conditions were detected in a mutant cell:

The cell is running out of ATP, while ADP is building up to very high levels. NADH is building up to very high levels, while the level of NAD⁺ is becoming very low.

The amount of protons in the intermembrane space and in the matrix is becoming more equal (the strength of the proton gradient is decreasing/weakening).

Use this information to predict which stage of cellular respiration is not functioning normally in this mutant cell.



- A. glycolysis
- B. citric acid cycle
- C. pyruvate oxidation
- D. electron transport chain
- E. ATP synthase

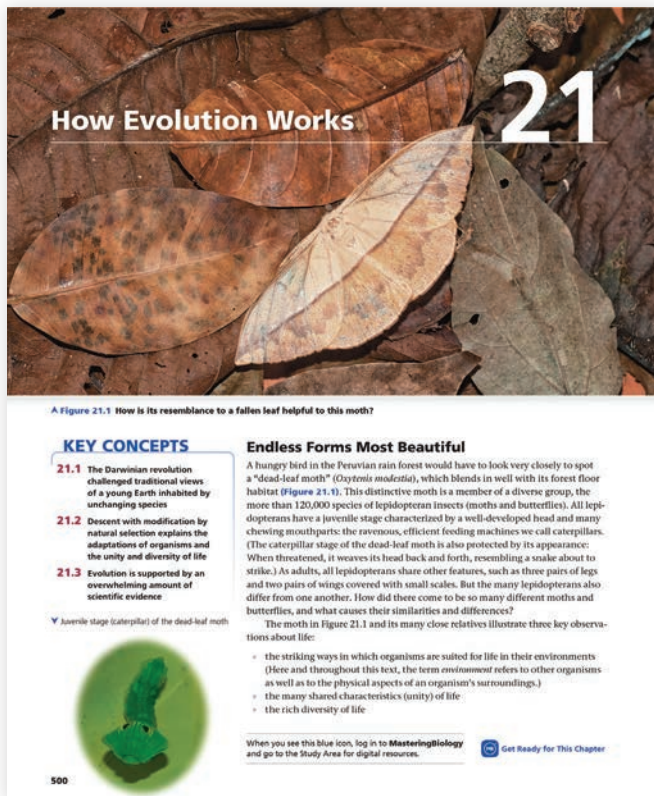


See the Big Picture

Each chapter is organized around a framework of 3 to 7 **Key Concepts** that focus on the big picture and provide a context for supporting details.

Every chapter opens with a visually dynamic **photo** accompanied by an **intriguing question** that invites students into the chapter.

The **List of Key Concepts** introduces the big ideas covered in the chapter.



After reading a Key Concept section, students can check their understanding using the **Concept Check Questions**.

Questions throughout the chapter encourage students to **read the text actively**.

What If? Questions ask students to apply what they've learned.

Make Connections Questions ask students to relate content in the chapter to material presented earlier in the course.

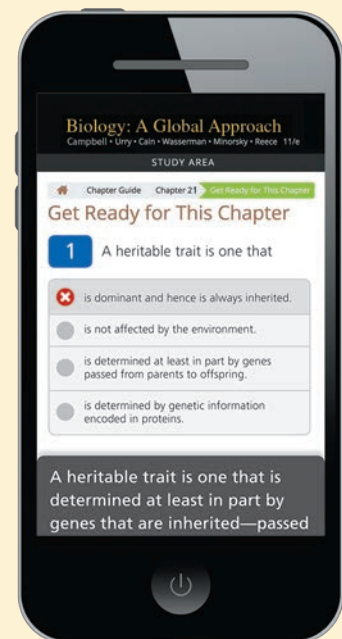
CONCEPT CHECK 21.2

1. How does the concept of descent with modification explain both the unity and diversity of life?
2. **WHAT IF? >** If you discovered a fossil of an extinct mammal that lived high in the Andes, would you predict that it would more closely resemble present-day mammals from South American jungles or present-day mammals that live high in Asian mountains? Explain.
3. **MAKE CONNECTIONS >** Review the relationship between genotype and phenotype (see Figures 14.5 and 14.6). Suppose that in a particular pea population, flowers with the white phenotype are favored by natural selection. Predict what would happen over time to the frequency of the *p* allele in the population, and explain your reasoning.

For suggested answers, see Appendix A.

Get Ready for This Chapter

NEW! Get Ready for This Chapter questions provide a quick check of students' knowledge of basic information needed to learn the new content of a chapter, with feedback.



These questions are available as MasteringBiology assignments and as self-study quizzes in the Study Area.

The **Summary of Key Concepts** refocuses students on the main points of the chapter.



VOCAB SELF-QUIZ
goo.gl/Rn5Uax



PRACTICE TEST
goo.gl/iASvgL

NEW! QR codes and URLs at the end of every chapter give students quick access to **Vocabulary Self-Quizzes and Practice Tests** on their smartphones, tablets, and computers.

21 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 21.1

The Darwinian revolution challenged traditional views of a young Earth inhabited by unchanging species (pp. 501–503)

- Darwin proposed that life's diversity arose from ancestral species through natural selection, a departure from prevailing views.
- Cuvier studied fossils but denied that evolution occurs; he proposed that sudden catastrophic events in the past caused species to disappear from an area.
- Hutton and Lyell thought that geologic change could result from gradual mechanisms that operated in the past in the same manner as they do today.
- Lamarck hypothesized that species evolve, but the underlying mechanisms he proposed are not supported by evidence.

1 Why was the age of Earth important for Darwin's ideas about evolution?

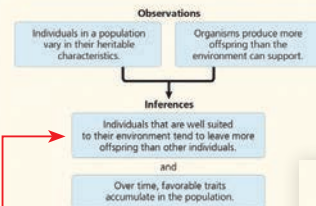
CONCEPT 21.2

Descent with modification by natural selection explains the adaptations of organisms and the unity and diversity of life (pp. 503–508)

- Darwin's experiences during the voyage of the *Beagle* gave rise to his idea that new species originate from ancestral forms through the accumulation of adaptations. He refined his theory for many years and finally published it in 1859 after learning that Wallace had come to the same idea.

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- In *The Origin of Species*, Darwin proposed that over long periods of time, descent with modification produced the rich diversity of life through the mechanism of natural selection.



2 Describe how overreproduction and heritable variation relate to evolution by natural selection.

CONCEPT 21.3

Evolution is supported by an overwhelming amount of scientific evidence (pp. 509–516)

- Researchers have directly observed natural selection leading to adaptive evolution in many studies, including research on soapberry bug populations and MRSA.

Summary of Key Concepts Questions check students' understanding of a key idea from each concept.

Summary Figures recap key information visually.

- Organisms share characteristics because of common descent (**homology**) or because natural selection affects independently evolving species in similar environments in similar ways (**convergent evolution**).
- Fossils show that past organisms differed from living organisms, that many species have become extinct, and that species have evolved over long periods of time; fossils also document the evolutionary origin of new groups of organisms.
- Evolutionary theory can explain some biogeographic patterns.

3 Summarize the different lines of evidence supporting the hypothesis that cetaceans descended from land mammals and are closely related to even-toed ungulates.

TEST YOUR UNDERSTANDING

Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

- 6. EVOLUTION CONNECTION** Explain why anatomical and molecular features often fit a similar nested pattern. In addition, describe a process that can cause this not to be the case.
- 7. SCIENTIFIC INQUIRY • DRAW IT** Mosquitoes resistant to the pesticide DDT first appeared in India in 1959, but now are found throughout the world. (a) Graph the data in the table below. (b) Examine the graph, then hypothesize why the percentage of mosquitoes resistant to DDT rose rapidly. (c) Suggest an explanation for the global spread of DDT resistance.

Month	0	8	12
Mosquitoes Resistant* to DDT	4%	45%	77%

*Mosquitoes were considered resistant if they were not killed within 1 hour of receiving a dose of 4% DDT.
Data from C. J. Curtis et al., Selection for and against insecticide resistance and possible methods of inhibiting the evolution of resistance in mosquitoes, *Ecological Entomology* 3:273–287 (1978).

8. WRITE ABOUT A THEME: INTERACTIONS Write a short essay (about 100–150 words) evaluating whether changes to an organism's physical environment are likely to result in evolutionary change. Use an example to support your reasoning.

9. SYNTHESIZE YOUR KNOWLEDGE



This honey pot ant (genus *Myrmecocystus*) can store liquid food inside its expandable abdomen. Consider other ants you are familiar with, and explain how a honey pot ant exemplifies three key features of life: adaptation, unity, and diversity.

For selected answers, see Appendix A.

For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Evolution, the fundamental theme of biology, is emphasized throughout. Every chapter has a section explicitly relating the chapter content to evolution:

Evolution of the Genetic Code

EVOLUTION The genetic code is nearly universal, shared by organisms from the simplest bacteria to the most complex plants and animals. The mRNA codon CCG, for instance, translated as the amino acid proline in all organisms whose genetic code has been examined. In laboratory experiments, genes can be transcribed and translated after being transplanted from one species to another, sometimes with striking results, as shown in **Figure 17.7**. Bacteria can be programmed by the insertion of human genes to synthesize certain human proteins for medical use, such as insulin. Applications have produced many exciting developments in the area of biotechnology (see Concept 19.4).

Figure 17.7 Evidence for evolution: expression of genes from different species. Because diverse forms of life share a common genetic code due to their shared ancestry, one species can be programmed to produce proteins characteristic of a second species by introducing DNA from the second species into the first.



(a) Tobacco plant expressing a jellyfish gene. The yellow glow is produced by a chemical reaction catalyzed by the protein product of the jellyfish gene.
(b) Pig expressing a jellyfish gene. Researchers injected a jellyfish gene for a fluorescent protein into fertilized pig eggs. One developed into this fluorescent pig.

Evolution Connection Questions are included in every Chapter Review.

Synthesize Your Knowledge Questions

ask students to apply their understanding of the chapter content to explain an intriguing photo.

Build Visual Skills

NEW! Visualizing Figures teach students how to interpret diagrams and models in biology. Embedded questions give students practice applying visual skills as they read the figure.

For more practice, each Visualizing Figure is accompanied by an automatically graded assignment in MasteringBiology with feedback for students.

Figure 22.5 Visualizing Phylogenetic Relationships

A phylogenetic tree visually represents a hypothesis of how a group of organisms are related. This figure explores how the way a tree is drawn conveys information.

Instructors: Additional questions related to this Visualizing Figure can be assigned in MasteringBiology.

Parts of a Tree

This tree shows how the five groups of organisms at the tips of the branches, called taxa, are related. Each branch point represents the common ancestor of the evolutionary lineages diverging from it.

This branch point represents the common ancestor of all the animal groups shown in this tree.

Each horizontal branch represents an evolutionary lineage. The length of the branch is arbitrary unless the diagram specifies that branch lengths represent information such as time or amount of genetic change (see Figure 22.13).

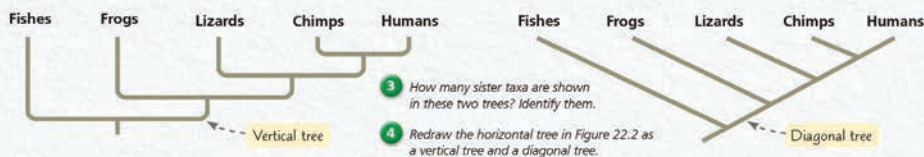
Each position along a branch represents an ancestor in the lineage leading to the taxon named at the tip.

- 1 According to this tree, which group or groups of organisms are most closely related to frogs?
- 2 Label the part of the diagram that represents the most recent common ancestor of frogs and humans.

Sister taxa are groups of organisms that share a common ancestor that is not shared by any other group. Chimps and humans are an example of sister taxa in this tree.

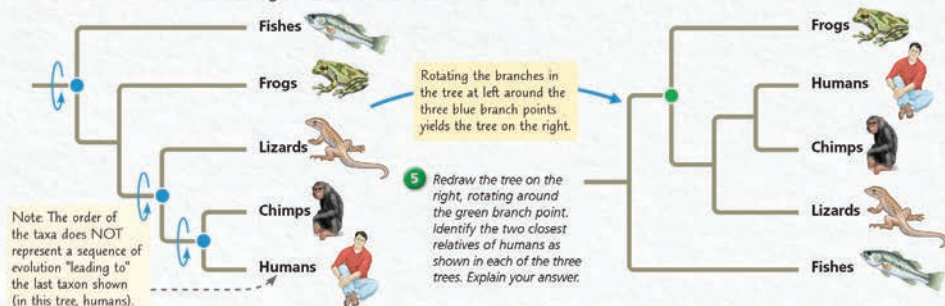
Alternative Forms of Tree Diagrams

These diagrams are referred to as "trees" because they use the visual analogy of branches to represent evolutionary lineages diverging over time. In this text, trees are usually drawn horizontally, as shown above, but the same tree can be drawn vertically or diagonally without changing the relationships it conveys.



Rotating Around Branch Points

Rotating the branches of a tree around a branch point does not change what they convey about evolutionary relationships. As a result, the order in which taxa appear at the branch tips is not significant. What matters is the branching pattern, which signifies the order in which the lineages have diverged from common ancestors.



NEW! Visual Skills Questions give students practice interpreting illustrations and photos in the text.

Visualizing Figures include:

Figure 5.16 Visualizing Proteins, p. 127

Figure 7.32 Visualizing the Scale of the Molecular Machinery in a Cell, pp. 192-193

Figure 16.7 Visualizing DNA, p. 369

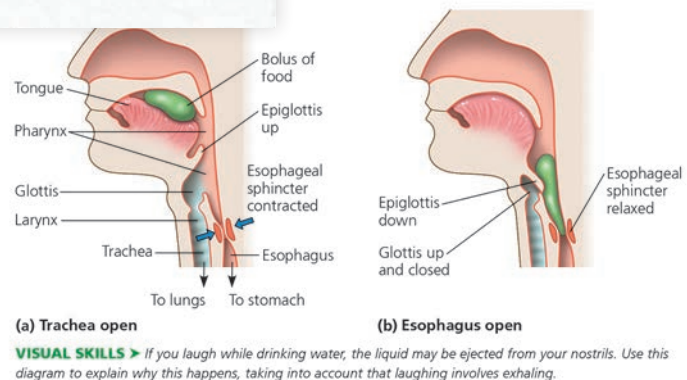
Figure 22.5 Visualizing Phylogenetic Relationships, shown at left and on p. 522

Figure 25.8 Visualizing the Scale of Geologic Time, pp. 586-587

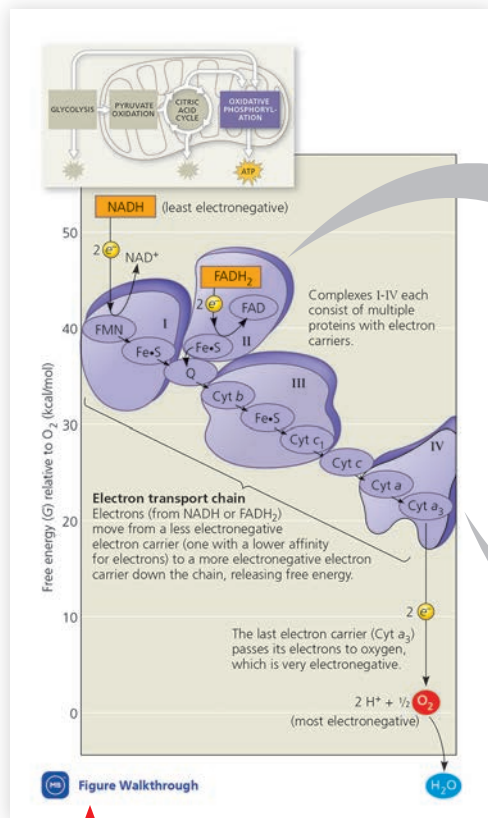
Figure 35.11 Visualizing Primary and Secondary Growth, p. 819

Figure 46.8 Visualizing Gastrulation, p. 1080

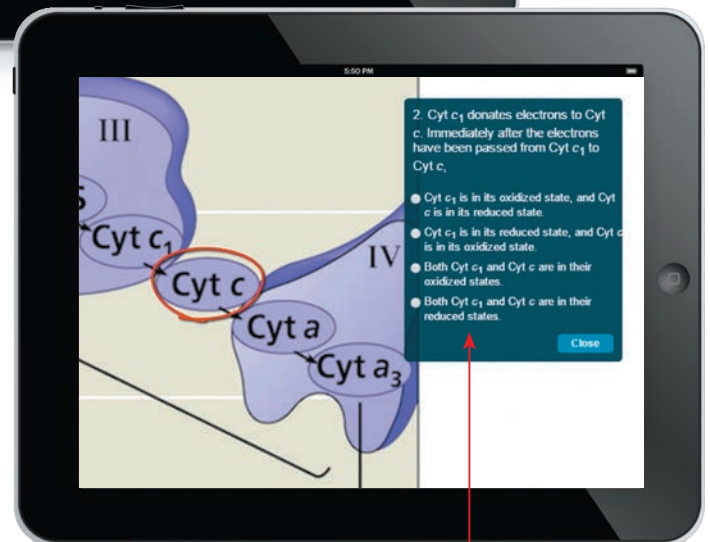
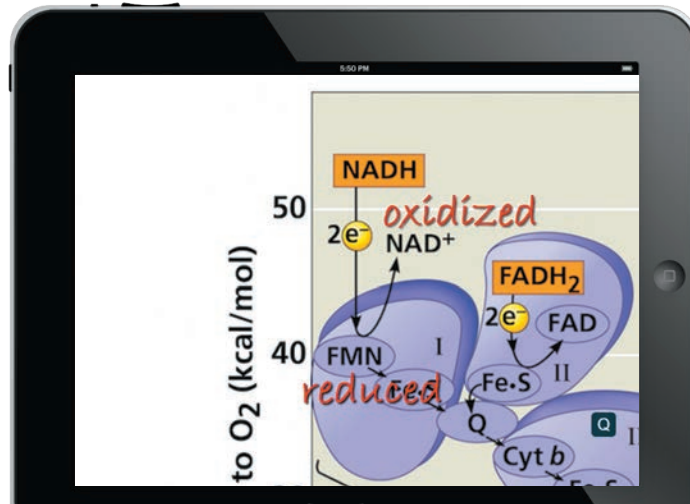
Figure 55.13 Visualizing Biogeochemical Cycles, p. 1305



NEW! Figure Walkthroughs guide students through key figures with narrated explanations, figure markups, and questions that reinforce important points.



A note in the print book lets students and instructors know when a Figure Walkthrough is available in the Study Area.



Questions embedded in each Figure Walkthrough encourage students to be active participants in their learning. The Figure Walkthroughs can also be assigned in MasteringBiology with higher-level questions.

Figure 2.17

Photosynthesis: a solar-powered rearrangement of matter. *Elodea*, a freshwater plant, produces sugar by rearranging the atoms of carbon dioxide and water in the chemical process known as photosynthesis, which is powered by sunlight. Much of the sugar is then converted to other food molecules. Oxygen gas (O_2) is a by-product of photosynthesis; notice the bubbles of O_2 gas escaping from the leaves submerged in water.

DRAW IT ➤ Add labels and arrows on the photo showing the reactants and products of photosynthesis as it takes place in a leaf.



EXPANDED! Draw It exercises give students practice creating visuals. Students are asked to put pencil to paper and draw a structure, annotate a figure, or graph experimental data.

Make Connections Visually

Eleven **Make Connections Figures** pull together content from different chapters, providing a visual representation of “big picture” relationships.

Make Connections Figures include:

Figure 5.26 Contributions of Genomics and Proteomics to Biology, p. 136

Figure 11.23 The Working Cell, pp. 280-281

Figure 18.27 Genomics, Cell Signaling, and Cancer, pp. 440-441

Figure 23.18 The Sickle-Cell Allele, shown at right and on pp. 556-557 →

Figure 33.9 Maximizing Surface Area, p. 747

NEW! Figure 37.10 Mutualism Across Kingdoms and Domains, p. 865

Figure 39.27 Levels of Plant Defenses Against Herbivores, pp. 920-921

Figure 40.23 Life Challenges and Solutions in Plants and Animals, pp. 946-947

Figure 44.17 Ion Movement and Gradients, p. 1043

Figure 55.19 The Working Ecosystem, pp. 1312-1313

NEW! Figure 56.30 Climate Change Has Effects at All Levels of Biological Organization, pp. 1336-1337

NEW! Media references integrated into the text

direct students to digital content in the MasteringBiology Study Area that will help them prepare for class and succeed on exams. Video resources include HHMI BioInteractive Short Films (documentary-quality movies from the Howard Hughes Medical Institute) and much more.

Figure 23.18 MAKE CONNECTIONS

The Sickle-Cell Allele

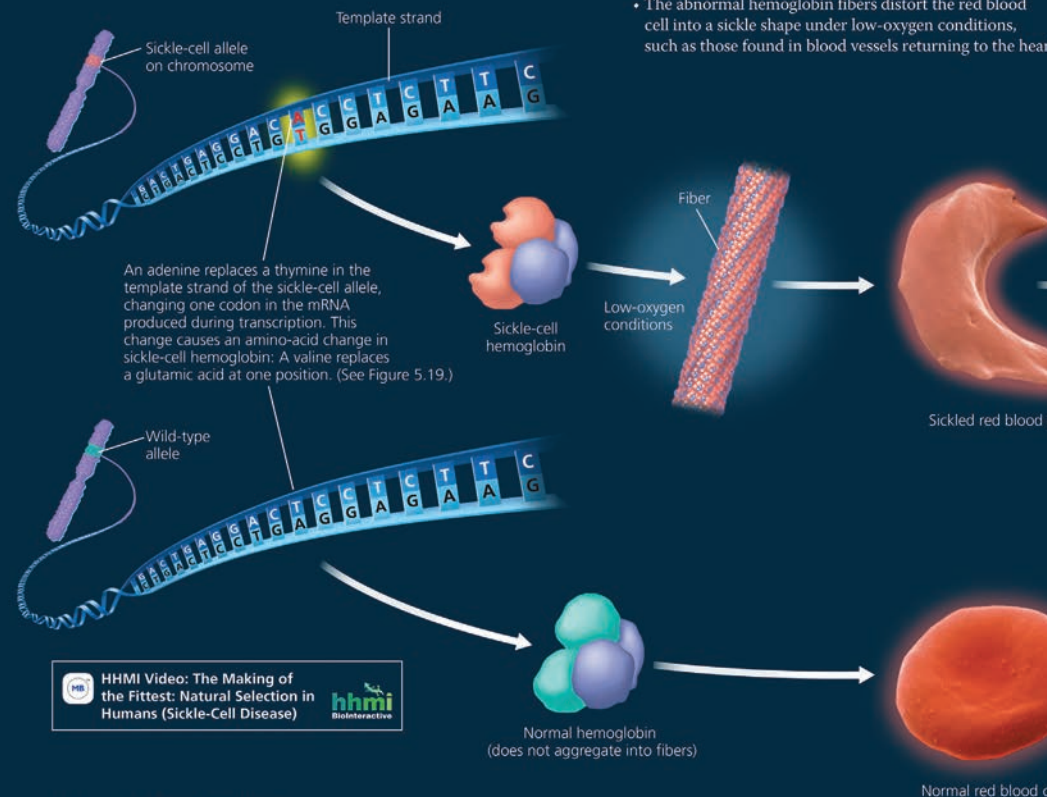
This child has sickle-cell disease, a genetic disorder that strikes individuals that have two copies of the sickle-cell allele. This allele causes an abnormality in the structure and function of hemoglobin, the oxygen-carrying protein in red blood cells. Although sickle-cell disease is lethal if not treated, in some regions the sickle-cell allele can reach frequencies as high as 15–20%. How can such a harmful allele be so common?

Events at the Molecular Level

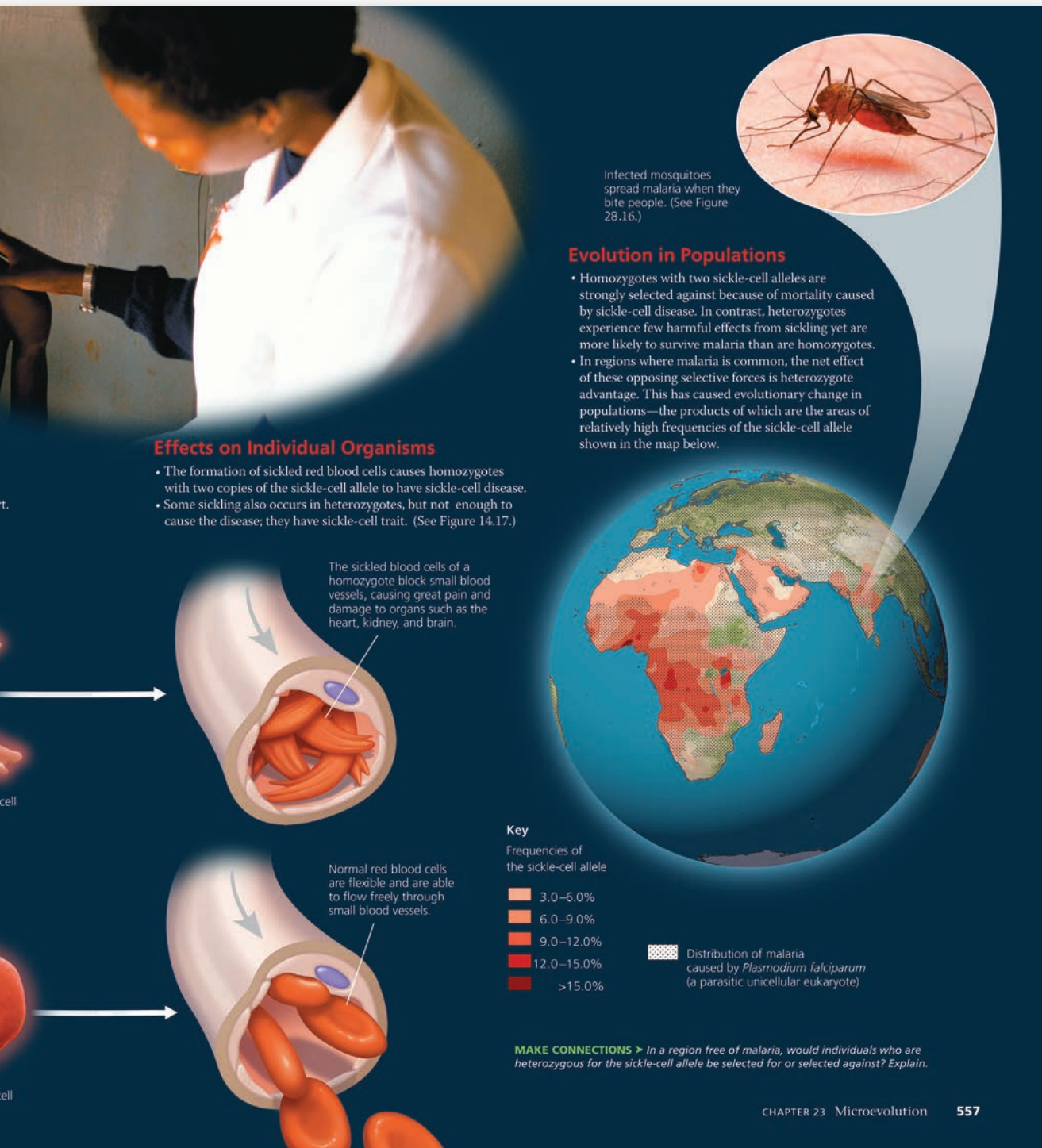
- Due to a point mutation, the sickle-cell allele differs from the wild-type allele by a single nucleotide. (See Figure 17.26.)
- The resulting change in one amino acid leads to hydrophobic interactions between the sickle-cell hemoglobin proteins under low-oxygen conditions.
- As a result, the sickle-cell proteins bind to each other in chains that together form a fiber.

Consequences for Cells

- The abnormal hemoglobin fibers distort the red blood cell into a sickle shape under low-oxygen conditions, such as those found in blood vessels returning to the heart.



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Make Connections Questions in every chapter ask students to relate content in the chapter to material presented earlier in the course.

Practice Scientific Skills

Scientific Skills Exercises use real data to build key skills needed for biology, including **data analysis**, **graphing**, **experimental design**, and **math skills**.

Each Scientific Skills Exercise is based on an **experiment related to the chapter content**.

Most Scientific Skills Exercises use **data from published research**, which is cited in the exercise.

Questions build in difficulty, walking students through new skills step by step and providing opportunities for higher-level critical thinking.

SCIENTIFIC SKILLS EXERCISE

Interpreting a Scatter Plot with Two Sets of Data

Is Glucose Uptake into Cells Affected by Age? Glucose, an important energy source for animals, is transported into cells by facilitated diffusion using protein carriers. In this exercise, you will interpret a graph with two sets of data from an experiment that examined glucose uptake over time in red blood cells from guinea pigs of different ages. You will determine if the cells' rate of glucose uptake depended on the age of the guinea pigs.

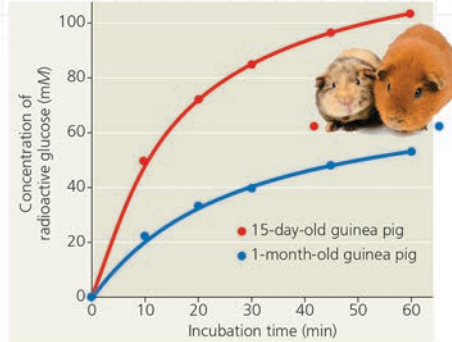
How the Experiment Was Done Researchers incubated guinea pig red blood cells in a 300 mM (millimolar) radioactive glucose solution at pH 7.4 at 25°C. Every 10 or 15 minutes, they removed a sample of cells and measured the concentration of radioactive glucose inside those cells. The cells came from either a 15-day-old or a 1-month-old guinea pig.

Data from the Experiment When you have multiple sets of data, it can be useful to plot them on the same graph for comparison. In the graph here, each set of dots (of the same color) forms a scatter plot, in which every data point represents two numerical values, one for each variable. For each data set, a curve that best fits the points has been drawn to make it easier to see the trends. (For additional information about graphs, see the Scientific Skills Review in Appendix F.)

INTERPRET THE DATA

1. First make sure you understand the parts of the graph. (a) Which variable is the independent variable—the variable controlled by the researchers? (b) Which variable is the dependent variable—the variable that depended on the treatment and was measured by the researchers? (c) What do the red dots represent? (d) The blue dots?
2. From the data points on the graph, construct a table of the data. Put "Incubation Time (min)" in the left column of the table.

Glucose Uptake over Time in Guinea Pig Red Blood Cells



Data from T. Kondo and E. Beutler, Developmental changes in glucose transport of guinea pig erythrocytes, *Journal of Clinical Investigation* 65:1–4 (1980).

3. What does the graph show? Compare and contrast glucose uptake in red blood cells from 15-day-old and 1-month-old guinea pigs.
4. Develop a hypothesis to explain the difference between glucose uptake in red blood cells from 15-day-old and 1-month-old guinea pigs. (Think about how glucose gets into cells.)
5. Design an experiment to test your hypothesis.

Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

All Scientific Skills Exercises are available as **interactive assignments in MasteringBiology** that are automatically graded.

SCIENTIFIC SKILLS EXERCISES are available for every chapter:

- | | |
|---|--|
| 1 Interpreting a Pair of Bar Graphs, p. 71 | 15 Using the Chi-Square (χ^2) Test, p. 354 |
| 2 Calibrating a Standard Radioactive Isotope Decay Curve and Interpreting Data, p. 81 | 16 Working with Data in a Table, p. 368 |
| 3 Interpreting a Scatter Plot with a Regression Line, p. 102 | 17 Interpreting a Sequence Logo, p. 401 |
| 4 Working with Moles and Molar Ratios, p. 106 | 18 Analyzing DNA Deletion Experiments, p. 423 |
| 5 Analyzing Polypeptide Sequence Data, p. 137 | 19 Analyzing Quantitative and Spatial Gene Expression Data* |
| 6 Making a Line Graph and Calculating a Slope, p. 155 | 20 Reading an Amino Acid Sequence Identity Table, p. 490 |
| 7 Using a Scale Bar to Calculate Volume and Surface Area of a Cell, p. 169 | 21 Making and Testing Predictions, p. 515 |
| 8 Interpreting a Scatter Plot with Two Sets of Data, shown above and on p. 206 | 22 Using Protein Sequence Data to Test an Evolutionary Hypothesis, p. 536 |
| 9 Using Experiments to Test a Model* | 23 Using the Hardy-Weinberg Equation to Interpret Data and Make Predictions, p. 547 |
| 10 Making a Bar Graph and Evaluating a Hypothesis, p. 251 | 24 Identifying Independent and Dependent Variables, Making a Scatter Plot, and Interpreting Data, p. 567 |
| 11 Making Scatter Plots with Regression Lines, p. 277 | 25 Estimating Quantitative Data from a Graph and Developing Hypotheses, p. 592 |
| 12 Interpreting Histograms, p. 300 | 26 Analyzing a Sequence-Based Phylogenetic Tree to Understand Viral Evolution, p. 621 |
| 13 Making a Line Graph and Converting Between Units of Data, p. 314 | 27 Calculating and Interpreting Means and Standard Errors, p. 642 |
| 14 Making a Histogram and Analyzing a Distribution Pattern, p. 333 | Making a Bar Graph and Interpreting the Data* |

Apply Scientific Skills to Solving Problems

NEW! Problem-Solving Exercises guide students in applying scientific skills and interpreting real data in the context of solving a real-world problem.

PROBLEM-SOLVING EXERCISE

Are you a victim of fish fraud?

When buying salmon, perhaps you prefer the more expensive wild-caught Pacific salmon (*Oncorhynchus* species) over farmed Atlantic salmon (*Salmo salar*). But studies reveal that about 40% of the time, you aren't getting the fish you paid for! Watch the video in the MasteringBiology Study Area for more information.



ABC News Video: Fake Fish in Stores and Restaurants

Instructors: A version of this Problem-Solving Exercise can be assigned in Chapter 5 of MasteringBiology. A more extensive investigation is in Chapter 22 of MasteringBiology.

In this exercise, you will investigate whether a piece of salmon has been fraudulently labeled.

Your Approach The principle guiding your investigation is that DNA sequences from within a species or from closely related species are more similar to each other than are sequences from more distantly related species.

Your Data You've been sold a piece of salmon labeled as coho salmon (*Oncorhynchus kisutch*). To see whether your fish was labeled correctly, you will compare a short DNA sequence from your sample to standard sequences from the same gene for three salmon species. The sequences are:

Sample labeled as <i>O. kisutch</i> (coho salmon)	5'-CGGCACCGCCCTAAGTCTCT-3'
Standard sequences	
Sequence for <i>O. kisutch</i> (coho salmon)	5'-AGGCACCGCCCTAAGTCTAC-3'
Sequence for <i>O. keta</i> (chum salmon)	5'-AGGCACCGCCCTGAGCCTAC-3'
Sequence for <i>Salmo salar</i> (Atlantic salmon)	5'-CGGCACCGCCCTAAGTCTCT-3'

Your Analysis

1. Scan along the standard sequences (*O. kisutch*, *O. keta*, and *S. salar*), base by base, circling any bases that do not match the sequence from your fish sample.
2. How many bases differ between (a) *O. kisutch* and your fish sample? (b) *O. keta* and the sample? (c) *S. salar* and the sample?
3. For each standard, what percentage of its bases are identical to your sample?
4. Based on these data alone, state a hypothesis for the species identity of your sample. What is your reasoning?

Problem-Solving Exercises include:

Ch. 5: Are you a victim of fish fraud? *Shown at left and on p. 137*

Ch. 9: Can a skin wound turn deadly? *p. 216*

Ch. 17: Are insulin mutations the cause of three infants' neonatal diabetes? *p. 409*

Ch. 24: Is hybridization promoting insecticide resistance in mosquitoes that transmit malaria? *p. 572*

Ch. 34: Can declining amphibian populations be saved by a vaccine? *p. 785*

Ch. 39: How will climate change impact crop productivity? *p. 915*

Ch. 41: Is thyroid regulation normal in this patient? *p. 962*

Ch. 55: Can an insect outbreak threaten a forest's ability to absorb CO₂ from the atmosphere? *p. 1301*

A version of each Problem-Solving Exercise can also be assigned in MasteringBiology.

28 Interpreting Comparisons of Genetic Sequences, *p. 647*

29 Making Bar Graphs and Interpreting Data, *p. 681*

30 Using Natural Logarithms to Interpret Data, *p. 691*

31 Interpreting Genomic Data and Generating Hypotheses, *p. 709*
Synthesizing Information from Multiple Data Sets*

32 Calculating and Interpreting Correlation Coefficients, *p. 730*

33 Understanding Experimental Design and Interpreting Data, *p. 752*

34 Determining the Equation of a Regression Line, *p. 803*

35 Using Bar Graphs to Interpret Data, *p. 814*

36 Calculating and Interpreting Temperature Coefficients, *p. 842*

37 Making Observations, *p. 864*

38 Using Positive and Negative Correlations to Interpret Data, *p. 886*

39 Interpreting Experimental Results from a Bar Graph, *p. 916*

40 Interpreting Pie Charts, *p. 944*

41 Designing a Controlled Experiment, *p. 966*

42 Interpreting Data from an Experiment with Genetic Mutants, *p. 992*

43 Making and Interpreting Histograms, *p. 1012*

44 Describing and Interpreting Quantitative Data, *p. 1031*

45 Making Inferences and Designing an Experiment, *p. 1060*

46 Interpreting a Change in Slope, *p. 1079*

47 Comparing Two Variables on a Common x-Axis, *p. 1119*

48 Interpreting Data Values Expressed in Scientific Notation, *p. 1138*

49 Designing an Experiment using Genetic Mutants, *p. 1151*

50 Interpreting a Graph with Log Scales, *p. 1192*

51 Making a Bar Graph and a Line Graph to Interpret Data, *p. 1218*

52 Testing a Hypothesis with a Quantitative Model, *p. 1234*

53 Using the Logistic Equation to Model Population Growth, *p. 1256*

54 Making a Bar Graph and a Scatter Plot, *p. 1273*

55 Interpreting Quantitative Data in a Table, *p. 1303*

56 Graphing Cyclic Data, *p. 1334*

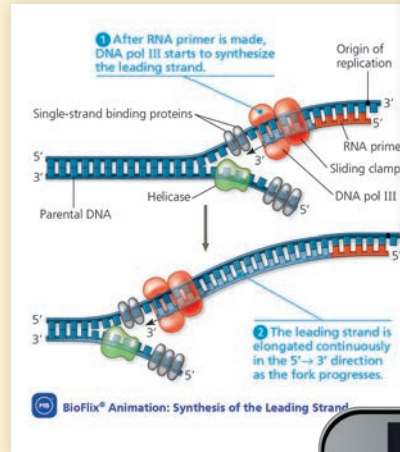
* Available only in MasteringBiology. All other Scientific Skills Exercises are in the print book, eText, and MasteringBiology.

Bring Biology to Life

NEW! Over 450 carefully chosen and edited **videos and animations** have been integrated into the print book, MasteringBiology Study Area, and eText at point of use to help students learn biology visually.

Media references in the print book direct students to digital resources in the Study Area and eText:

- **NEW!** Get Ready for This Chapter questions
- **NEW!** Figure Walkthroughs
- **NEW!** HHMI BioInteractive Videos and Animations
- **NEW!** QR codes and URLs for easy access to Vocabulary Self-Quizzes and Practice Tests
- BioFlix Animations
- ABC News Videos
- Campbell Interviews *and much more*



In April 1953, Watson and Crick surprised the scientific world with a succinct, one-page paper that reported their molecular model for DNA: the double helix, which has since become the symbol of molecular biology. Watson and Crick, along with Maurice Wilkins, were awarded the Nobel Prize in 1962 for this work. (Sadly, Rosalind Franklin had died at the age of 37 in 1958 and was thus ineligible for the prize.) The beauty of the double helix model was that the structure of DNA suggested the basic mechanism of its replication.

HHMI Video: Great Discoveries in Science: The Double Helix

hhmi
BioInteractive



Access the complete textbook online! The Campbell eText includes powerful interactive and customization functions, such as instructor and student note-taking, highlighting, bookmarking, search, and links to glossary terms.

Succeed with MasteringBiology

MasteringBiology improves results by engaging students before, during, and after class.

Before Class

◀ **Dynamic Study Modules** provide students with multiple sets of questions with extensive feedback so that they can **test, learn, and retest** until they achieve mastery of the textbook material.

NEW! Get Ready for This Chapter quizzes help students review content they need to understand from previous chapters (see p. 10).

Pre-Class Reading Quizzes help students pinpoint concepts that they understand and concepts that they need to review.

During Class

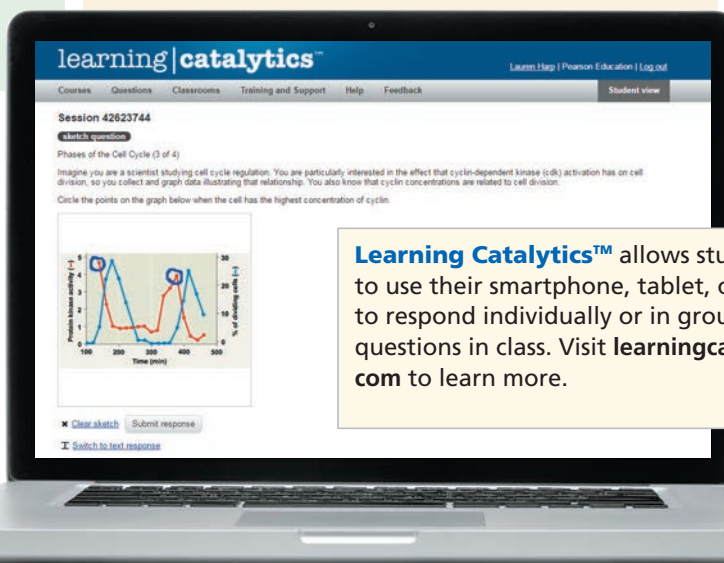
NEW! For ideas for in-class activities, see the **Ready-to-Go Teaching Modules** (see p. 9).



After Class

Hundreds of self-paced tutorials and coaching activities provide students with individualized coaching with specific hints and feedback on the toughest topics in the course.

Optional Adaptive Follow-up Assignments are based on each student's performance on the original MasteringBiology assignment and provide additional questions and activities tailored to each student's needs.

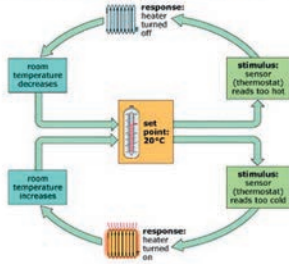


Learning Catalytics™ allows students to use their smartphone, tablet, or laptop to respond individually or in groups to questions in class. Visit learningcatalytics.com to learn more.

Personalized Coaching in MasteringBiology

Part A - Maintaining homeostasis

An animal's body maintains a relatively constant internal environment. How is this accomplished? It is surprisingly similar to the way a thermostat and heating system maintain a relatively constant temperature inside a room. The diagram below shows how a thermostat responds when the temperature becomes too hot or too cold.



Adapted from Biology by Campbell and Reece © Pearson Education, Inc.

Drag the terms on the left to the appropriate blanks on the right to complete the sentences. Not all terms will be used.

negative

decreases

1. This heating system maintains room temperature at or near a particular value, known as the **set point**.
2. **When open the window, and** blast of icy air enters the room. The temperature drops to 17 degrees Celsius, which acts as a **stimulus** to the heating system.
3. The thermostat is a **sensor** that detects the stimulus and triggers a response.
4. The heater turns on, and the temperature in the room **increases** until it returns to the original setting.
5. The response of the heating system reduces the stimulus. This is an example of **positive** feedback.
6. The way this heating system maintains a stable room temperature is similar to the way an animal's body controls many aspects of its internal environment. The maintenance of a relatively constant internal environment is known as **homeostasis**.

Submit [Hints](#) [My Answers](#) [Give Up](#) [Review Part](#)

Incorrect; Try Again; 5 attempts remaining

You filled in 1 of 6 blanks incorrectly. For sentence 5, you may want to review positive and negative feedback in Hint 2.

Hint 2. What is the difference between positive and negative feedback?

Let's take a closer look at positive and negative feedback.

Drag each statement into the appropriate bin depending on whether it applies to positive feedback or negative feedback.

the response to a stimulus reduces the stimulus

the response to a stimulus amplifies the stimulus

example: icy cold draft causes room heater to turn on

example: response to low blood glucose raises blood glucose

example: stronger and stronger contractions during childbirth

example: response to high blood glucose lowers blood glucose

positive feedback

negative feedback

Submit [My Answers](#) [Give Up](#)

1. If a student gets stuck...
2. Specific wrong-answer **feedback** appears in the purple feedback box.

3. **Hints** coach students to the correct response.
4. Optional **Adaptive Follow-Up Assignments** are based on the original homework assignment and provide additional coaching and practice as needed.

Question sets in the Adaptive Follow-Up Assignments **continuously adapt** to each student's needs, making efficient use of study time.

Homework: Animal Structure & Function

Due: 11:59pm on Thursday, June 16, 2016

You will receive no credit for items you complete after the assignment is due. [Grading Policy](#)

✓ You completed this assignment. [Start the Adaptive Follow-Up Now.](#)



Homework: Animal Structure & Function

Adaptive Follow-Up

Due: 11:59pm on Saturday, June 18, 2016

Parent Assignment: [Homework: Animal Structure & Function](#)
Question Sets: 3

This Adaptive Follow-Up assignment is designed specifically for you based on your performance on the Parent assignment. The system analyzes your responses and personalizes each question set to focus your studies and help you succeed. [Learn more](#)

You will receive no credit for items you complete after the assignment is due. [Grading Policy](#)

Congratulations! You tested out of this Adaptive Follow-Up.

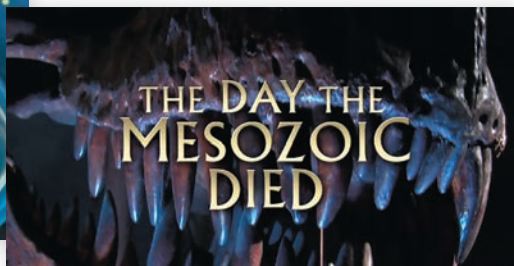
You received all 10 points for this Adaptive Follow-Up by scoring at or above the Test Out score on the Parent assignment. To learn more about the Test Out score, see the Grading Policy.

For additional study materials, please visit the [Study Area](#).

MasteringBiology offers thousands of tutorials, activities, and questions that can be assigned as homework. A few examples are shown below.



BioFlix Tutorials use 3-D, movie-quality animations and coaching exercises to help students master tough topics outside of class. Animations are also available in the Study Area and eText, and can be shown in class.



EXPANDED! HHMI BioInteractive Short Films, documentary-quality movies from the Howard Hughes Medical Institute, engage students in topics from the discovery of the double helix to evolution, with assignable questions.

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Gradebook

Filter* Showing Score in All Categories for All Students

Score Time Difficulty

Students per page: 25

NAME	Intro. SP	Chapter 5	Lab 2	CH5	CH5 Ad. Up	Lab 3	CH5 HW	CH5 H. Up	Lab 4	TOTAL
Assigned Points	3	20	13	7	5	7	37	5	19	134
Class Average	45.5	82.8	88.1	84.0	86.7	91.8	85.0	90.0	31.6	
Laet01, Fred...	55.0	83.0	100	100	5.0	95.0	100	100	43.6	
Laet02, Fred...	48.7	82.0	90.0	100	80.2	72.9	80.5	80.0	32.8	
Laet03, Fred...	34.5	81.0	104	100	94.9	85.0	100	95.0	31.6	
Laet04, Fred...	40.2	0.0	34.3	83.7	65.3	00.0	0.0	00.0	27.9	
Laet05, Fred...	52.6	78.0	90.0	100	85.2	82.5	97.8	85.0	34.7	
Laet07, Fred...	50.0	51.0	101	100	85.9	90.0	96.1	95.0	31.8	
Laet08, Fred...	53.6	92.9	100	100	100	95.0	100	100	41.6	
Laet09, Fred...	52.6	78.0	104	100	90.8	78.3	100	95.0	36.1	
Laet10, Fred...	52.6	78.0	100	100	94.9	92.1	94.6	100	30.4	
Laet11, Fred...	62.7	78.2	100	100	92.8	100	100	100	32.6	
Laet12, Fred...	53.6	68.0	97.7	100	90.0	100	100	100	32.6	
Laet14, Fred...	53.6	74.4	85.3	89.7	89.3	95.8	100	100	30.8	
Laet15, Fred...	52.6	82.2	100	100	100	100	100	100	32.8	

Student scores on the optional **Adaptive Follow-Up Assignments** are recorded in the gradebook and offer additional diagnostic information for instructors to monitor learning outcomes and more.

Student and Lab Supplements

For Students

Spanish Glossary for Biology

By Laura P. Zanello, University of California, Riverside

978-0-32183498-0 / 0-321-83498-4

This resource provides definitions in Spanish for the glossary terms.

Into The Jungle: Great Adventures in the Search for Evolution

by Sean B. Carroll, University of Wisconsin, Madison

978-0-32155671-4 / 0-321-55671-2

These nine short tales vividly depict key discoveries in evolutionary biology and the excitement of the scientific process.

Get Ready for Biology

by Lori K. Garrett, Parkland College

978-0-32150057-1 / 0-321-50057-1

This engaging workbook helps students brush up on important math and study skills and get up to speed on biological terminology and the basics of chemistry and cell biology. Also available in MasteringBiology.

A Short Guide to Writing About Biology, Ninth Edition

by Jan A. Pechenik, Tufts University

978-1-292-12083-6 / 1-292-12083-5

This best-selling writing guide teaches students to think as biologists and to express ideas clearly and concisely through their writing.

For Lab

Investigating Biology Laboratory Manual, Eighth Edition

by Judith Giles Morgan, Emory University, and
M. Eloise Brown Carter, Oxford College of Emory University

978-1-292-06130-6 / 1-292-06130-8

With its distinctive investigative approach to learning, this best-selling laboratory manual is now more engaging than ever, with full-color art and photos throughout. The lab manual encourages students to participate in the process of science and develop creative and critical-reasoning skills.

Preparation Guide for Investigating Biology

Contains materials lists, suggested vendors, instructions for preparing solutions and constructing materials, schedules for planning advance preparation, and more. Available for downloading through the "Instructor Resources" area of MasteringBiology.

MasteringBiology® Virtual Labs is an online environment that promotes critical thinking skills using virtual experiments and explorations that can supplement or substitute for existing wet labs in microscopy, molecular biology, genetics, ecology, and systematics.

Instructor Resources

Instructor's Resource Materials for Biology, Eleventh Edition

The Instructor's Resource Materials consist of multiple sets of assets for each chapter. Specific features include:

- Editable figures (art and photos) and tables from the text in PowerPoint®
- Prepared PowerPoint Lecture Presentations for each chapter with lecture notes, editable figures (art and photos), tables
- JPEG images, including labeled and unlabeled art, photos from the text, and extra photos
- Clicker Questions in PowerPoint
- Test Bank questions in TestGen® software and Microsoft® Word
- Digital Transparencies

The Instructor Resources area of MasteringBiology includes:

- **NEW!** Ready-to-Go Teaching Modules help instructors efficiently make use of the available teaching tools for the toughest topics. Before-class assignments, in-class activities, and after-class assignments are provided for ease of use. Instructors can incorporate active learning into their course with the suggested activity ideas and clicker questions or Learning Catalytics questions.
- Editable figures (art and photos) and tables from the text in PowerPoint
- Prepared PowerPoint Lecture Presentations for each chapter with lecture notes, editable figures (art and photos), tables, and links to animations and videos
- JPEG images, including labeled and unlabeled art, photos from the text, and extra photos
- Clicker Questions in PowerPoint
- 400 instructor animations and videos, including BioFlix 3-D animations and ABC News Videos
- Test Bank (in Word and TestGen)
- Digital Transparencies
- Answers to Scientific Skills Exercises, Problem-Solving Exercises, Interpret the Data Questions, and Essay Questions; includes Rubric and Tips for Grading Short-Answer Essays
- Instructor Guides for Supplements: Investigating Biology Lab Prep Guide and Investigating Biology Lab Data Tables
- Quick Reference Guide

Test Bank for Biology, Eleventh Edition

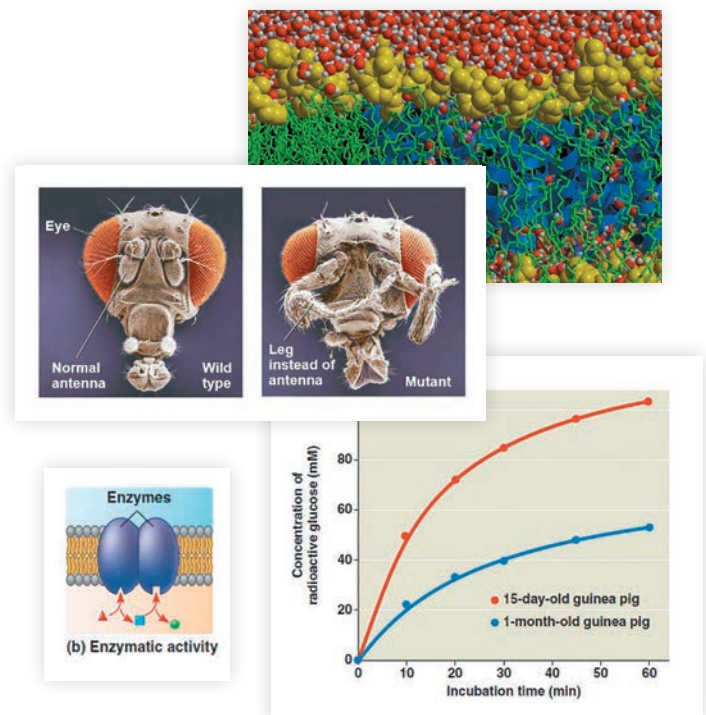
978-1-292-17048-0 / 1-292-17048-4

This invaluable resource contains over 4,500 questions, including scenario-based questions and art, graph, and data interpretation questions. The Test Bank is available through the "Instructor Resources" area of MasteringBiology.

Overview: Orchestrating Life's Processes

- The development of a fertilized egg into an adult requires a precisely regulated program of gene expression
- Understanding this program has progressed mainly by studying **model organisms**
- Stem cells are key to the developmental process
- Orchestrating proper gene expression by all cells is crucial for life

▲ **Customizable PowerPoint Lectures** provide a jumpstart for instruction.



▲ **All of the art, graphs, and photos from the text** are provided with customizable labels. More than 1,600 photos from the text and other sources are included.

Who conducted the X-ray diffraction studies that were key to the discovery of the structure of DNA?

- A. Griffith
- B. **Franklin**
- C. Meselson and Stahl
- D. Chargaff
- E. McClintock

One reason that deserts tend to be found near 30° north and south latitudes is that

- A. deserts are dry.
- B. it's warmer near the equator.
- C. global air circulation and precipitation patterns affect where rain falls.
- D. desert soils are different from tropical rain forest soils.
- E. mountains change rainfall patterns.

▲ **Clicker Questions** can be used to stimulate effective classroom discussions (for use with or without clickers).

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[†]A related Experimental Inquiry Tutorial can be assigned in MasteringBiology.®

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Shirley Tilghman

Princeton University

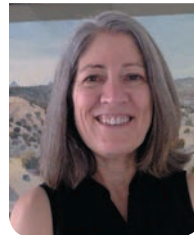
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Jack Szostak

Harvard University

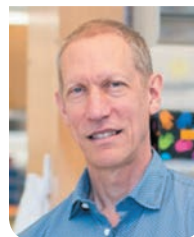
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Tracy Langkilde

Penn State University

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Interviews with prominent scientists have been a hallmark of *CAMPBELL BIOLOGY* since its inception, and conducting these interviews was again one of the great pleasures of revising the book. To open the eight units of this edition, we are proud to include interviews with Lovell Jones, Elba Serrano, Shirley Tilghman, Jack Szostak, Nancy Moran, Philip Benfey, Harald zur Hausen, and Tracy Langkilde.

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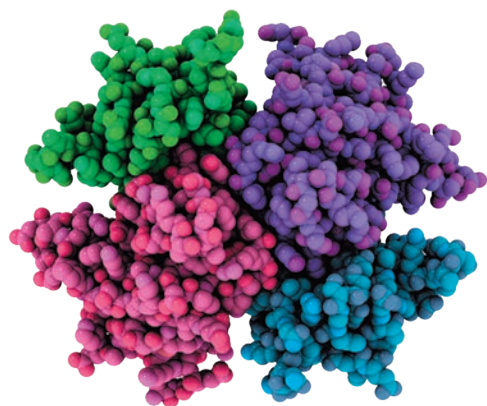
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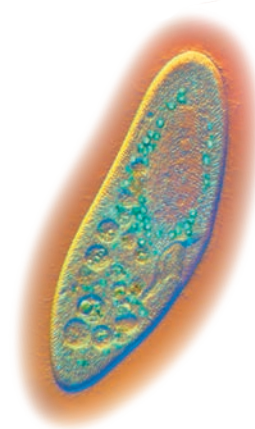
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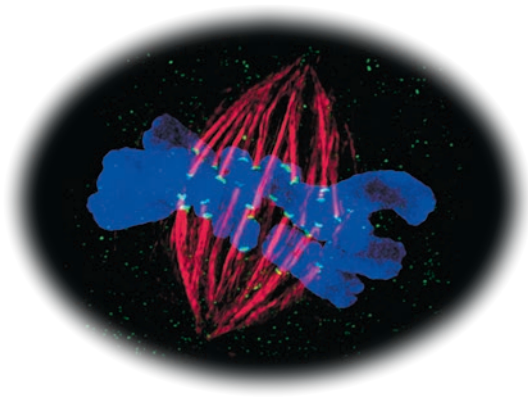
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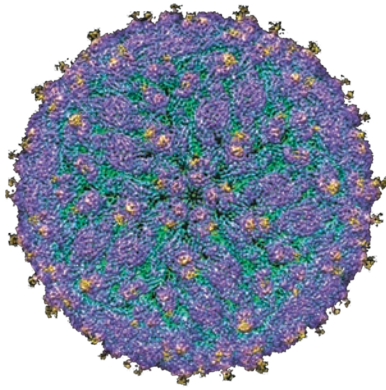
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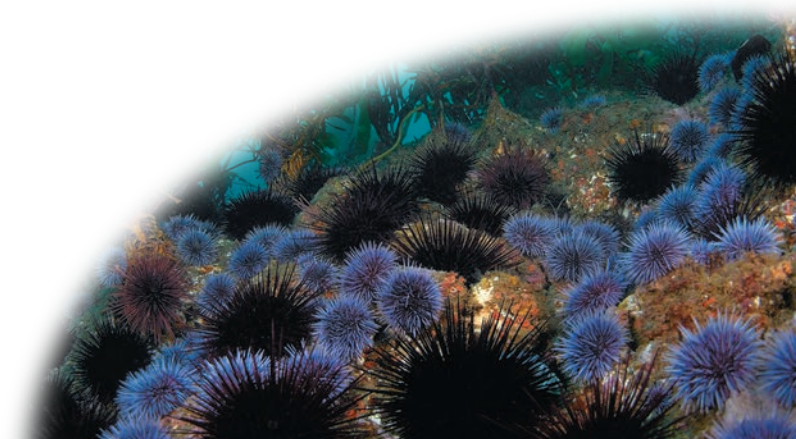
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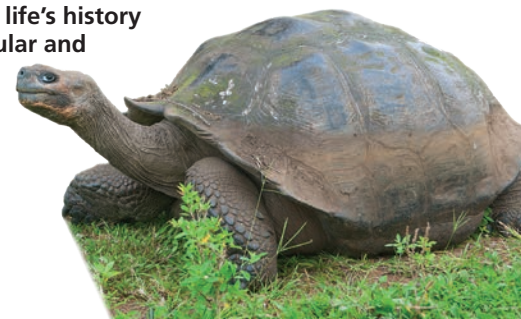
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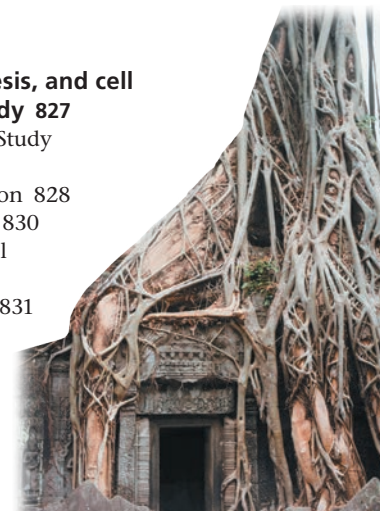
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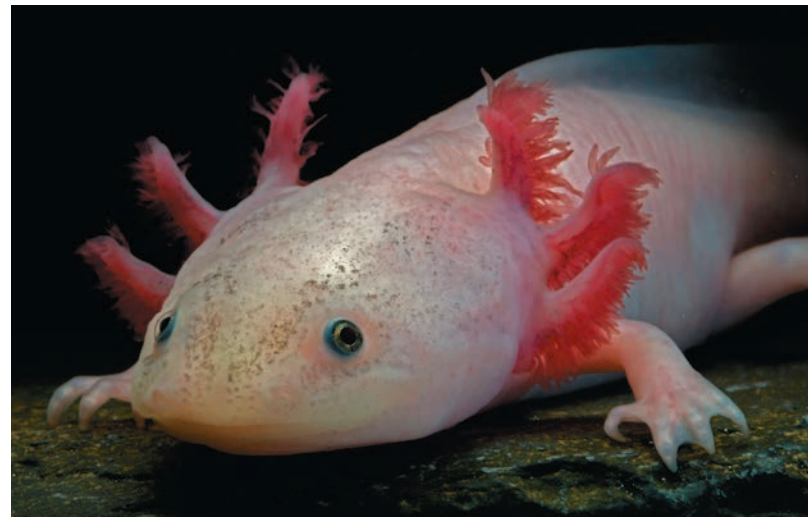
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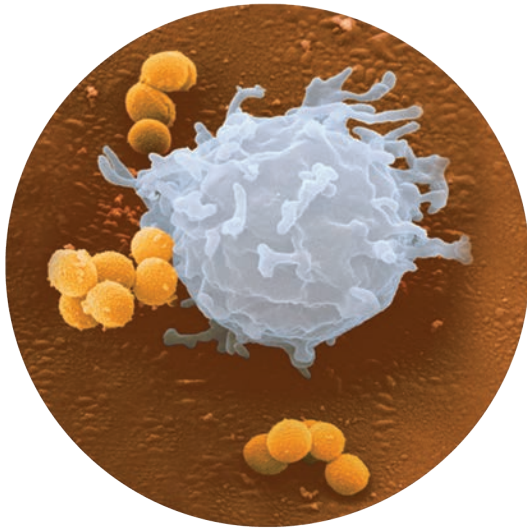
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Biology and Its Themes

1



▲ **Figure 1.1** What can this beach mouse (*Peromyscus polionotus*) teach us about biology?

KEY CONCEPTS

- 1.1** The study of life reveals unifying themes
- 1.2** The Core Theme: Evolution accounts for the unity and diversity of life
- 1.3** In studying nature, scientists make observations and form and test hypotheses
- 1.4** Science benefits from a cooperative approach and diverse viewpoints



▲ **An inland mouse of the species *Peromyscus polionotus*.** This mouse has a much darker back, side, and face than mice of the same species that inhabit sand dunes.

Inquiring About Life

There are few hiding places for a mouse among the sparse clumps of beach grass that dot the brilliant white sand dunes along the Florida seashore. However, the beach mice that live there have light, dappled fur, allowing them to blend into their surroundings (**Figure 1.1**). Mice of the same species (*Peromyscus polionotus*) also inhabit nearby inland areas. These mice are much darker in color, as are the soil and vegetation where they live (see smaller photo). For both beach mice and inland mice, the close color match of coat (fur) and environment is vital for survival, since hawks, herons, and other sharp-eyed predators periodically scan the landscape for prey. How has the color of each group of mice come to be so well matched, or *adapted*, to the local background?

An organism's adaptations to its environment, such as the mouse's protective camouflage, are the result of *evolution*, the process of change over time that has resulted in the astounding array of organisms found on Earth. Evolution is the fundamental principle of biology and the core theme of this book.

Although biologists know a great deal about life on Earth, many mysteries remain. Posing questions about the living world and seeking answers through scientific inquiry are the central activities of **biology**, the scientific study of life. Biologists' questions can be ambitious. They may ask how a single tiny cell

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Get Ready for This Chapter

becomes a tree or a dog, how the human mind works, or how the different forms of life in a forest interact. When questions occur to you as you observe the natural world, you are thinking like a biologist. More than anything else, biology is a quest, an ongoing inquiry about the nature of life.

At the most fundamental level, we may ask: What is life? Even a child realizes that a dog or a plant is alive, while a rock or a car is not. Yet the phenomenon we call life defies a simple, one-sentence definition. We recognize life by what living things do. **Figure 1.2** highlights some of the properties and processes we associate with life.

While limited to a handful of images, Figure 1.2 reminds us that the living world is wondrously varied. How do biologists make sense of this diversity and complexity? This opening chapter sets up a framework for answering this question. The first part of the chapter provides a panoramic view of the biological “landscape,” organized around some unifying themes. We then focus on biology’s core theme, evolution, which accounts for life’s unity and diversity. Next, we look at scientific inquiry—how scientists ask and attempt to answer questions about the natural world. Finally, we address the culture of science and its effects on society.

▼ **Figure 1.2** Some properties of life.

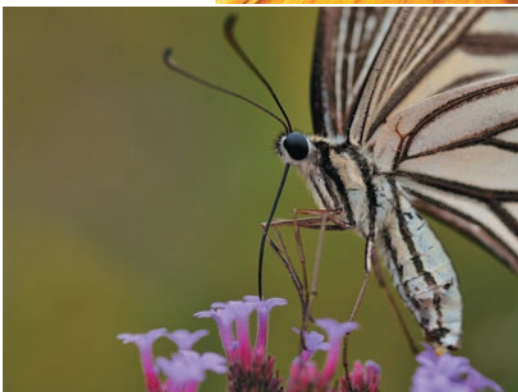
▼ **Order.** This close-up of a sunflower illustrates the highly ordered structure that characterizes life.



▲ **Evolutionary adaptation.** The overall appearance of this pygmy sea horse camouflages the animal in its environment. Such adaptations evolve over countless generations by the reproductive success of those individuals with heritable traits that are best suited to their environments.



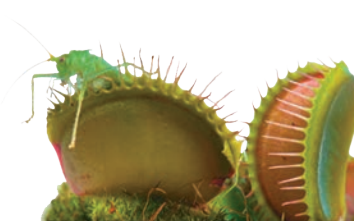
▲ **Regulation.** The regulation of blood flow through the blood vessels of this jackrabbit's ears helps maintain a constant body temperature by adjusting heat exchange with the surrounding air.



▲ **Energy processing.** This butterfly obtains fuel in the form of nectar from flowers. The butterfly will use chemical energy stored in its food to power flight and other work.



▲ **Growth and development.** Inherited information carried by genes controls the pattern of growth and development of organisms, such as this oak seedling.



▲ **Response to the environment.** The Venus flytrap on the left closed its trap rapidly in response to the environmental stimulus of a grasshopper landing on the open trap.



▼ **Reproduction.** Organisms (living things) reproduce their own kind.

CONCEPT 1.1

The study of life reveals unifying themes

Biology is a subject of enormous scope, and exciting new biological discoveries are being made every day. How can you organize into a comprehensible framework all the information you'll encounter as you study the broad range of topics included in biology? Focusing on a few big ideas will help.

Here are five unifying themes—ways of thinking about life that will still hold true decades from now.

- Organization
- Information
- Energy and Matter
- Interactions
- Evolution

In this section and the next, we'll briefly define and explore each theme.

▼ Figure 1.3 Exploring Levels of Biological Organization

◀ 1 The Biosphere

Even from space, we can see signs of Earth's life—in the green mosaic of the forests, for example. We can also see the entire **biosphere**, which consists of all life on Earth and all the places where life exists: most regions of land, most bodies of water, the atmosphere to an altitude of several kilometers, and even sediments far below the ocean floor.



◀ 2 Ecosystems

Our first scale change brings us to a North American mountain meadow, which is an example of an ecosystem, as are tropical forests, grasslands, deserts, and coral reefs. An **ecosystem** consists of all the living things in a particular area, along with all the nonliving components of the environment with which life interacts, such as soil, water, atmospheric gases, and light.



▶ 3 Communities

The array of organisms inhabiting a particular ecosystem is called a biological **community**. The community in our meadow ecosystem includes many kinds of plants, various animals, mushrooms and other fungi, and enormous numbers of diverse microorganisms, such as bacteria, that are too small to see without a microscope. Each of these forms of life belongs to a *species*—a group whose members can only reproduce with other members of the group.



▶ 4 Populations

A **population** consists of all the individuals of a species living within the bounds of a specified area. For example, our meadow includes a population of lupine (some of which are shown here) and a population of mule deer. A community is therefore the set of populations that inhabit a particular area.



▲ 5 Organisms

Individual living things are called **organisms**. Each plant in the meadow is an organism, and so is each animal, fungus, and bacterium.

Theme: New Properties Emerge at Successive Levels of Biological Organization

ORGANIZATION The study of life on Earth extends from the microscopic scale of the molecules and cells that make up organisms to the global scale of the entire living planet. As biologists, we can divide this enormous range into different levels of biological organization. In **Figure 1.3**, we zoom in from space to take a closer and closer look at life in a mountain meadow. This journey, depicted as a series of numbered steps, highlights the hierarchy of biological organization.

Zooming in through the levels of the biological hierarchy at ever-finer resolution illustrates an approach called *reductionism*. This method is so named because it reduces complex systems to simpler components that are more manageable to study. Reductionism is a powerful strategy in biology. For example, by studying the molecular structure of DNA that had been extracted from cells, James Watson and Francis Crick inferred the chemical basis of biological inheritance. Reductionism has propelled many major discoveries, but it provides a necessarily incomplete view of life on Earth, as we'll discuss next.

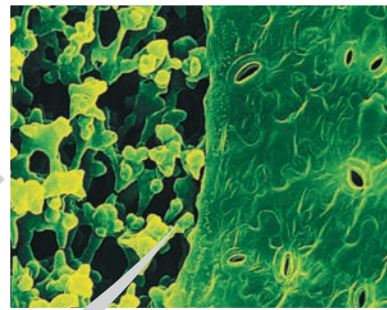
▼ 6 Organs

The structural hierarchy of life continues to unfold as we explore the architecture of a complex organism. A leaf is an example of an **organ**, a body part that is made up of multiple tissues and has specific functions in the body. Leaves, stems, and roots are the major organs of plants. Within an organ, each tissue has a distinct arrangement and contributes particular properties to organ function.



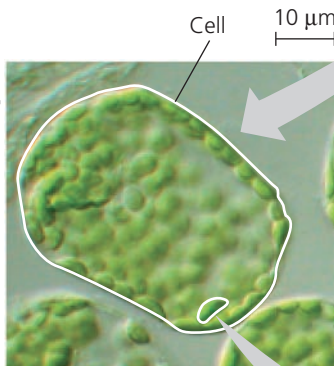
▼ 7 Tissues

Viewing the tissues of a leaf requires a microscope. Each **tissue** is a group of cells that work together, performing a specialized function. The leaf shown here has been cut on an angle. The honeycombed tissue in the interior of the leaf (left side of photo) is the main location of photosynthesis, the process that converts light energy to the chemical energy of sugar. The jigsaw puzzle-like "skin" on the surface of the leaf is a tissue called epidermis (right side of photo). The pores through the epidermis allow entry of the gas CO_2 , a raw material for sugar production.



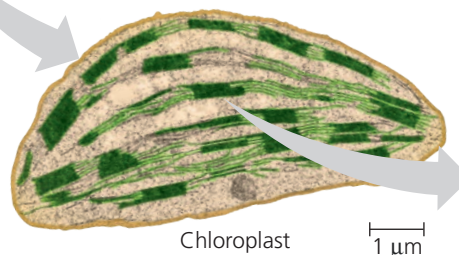
► 8 Cells

The **cell** is life's fundamental unit of structure and function. Some organisms consist of a single cell, which performs all the functions of life. Other organisms are multicellular and feature a division of labor among specialized cells. Here we see a magnified view of a cell in a leaf tissue. This cell is about 40 micrometers (μm) across—about 500 of them would reach across a small coin. Within these tiny cells are even smaller green structures called chloroplasts, which are responsible for photosynthesis.



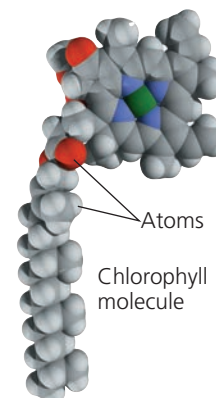
▼ 9 Organelles

Chloroplasts are examples of **organelles**, the various functional components present in cells. The image below, taken by a powerful microscope, shows a single chloroplast.



▼ 10 Molecules

Our last scale change drops us into a chloroplast for a view of life at the molecular level. A **molecule** is a chemical structure consisting of two or more units called atoms, represented as balls in this computer graphic of a chlorophyll molecule.



Chlorophyll is the pigment that makes a leaf green, and it absorbs sunlight during photosynthesis. Within each chloroplast, millions of chlorophyll molecules are organized into systems that convert light energy to the chemical energy of food.

Emergent Properties

Let's reexamine Figure 1.3, beginning this time at the molecular level and then zooming out. This approach allows us to see novel properties emerge at each level that are absent from the preceding one. These **emergent properties** are due to the arrangement and interactions of parts as complexity increases. For example, although photosynthesis occurs in an intact chloroplast, it will not take place in a disorganized test-tube mixture of chlorophyll and other chloroplast molecules. The coordinated processes of photosynthesis require a specific organization of these molecules in the chloroplast. Isolated components of living systems—the objects of study in a reductionist approach—lack a number of significant properties that emerge at higher levels of organization.

Emergent properties are not unique to life. A box of bicycle parts won't transport you anywhere, but if they are arranged in a certain way, you can pedal to your chosen destination. Compared with such nonliving examples, however, biological systems are far more complex, making the emergent properties of life especially challenging to study.

To fully explore emergent properties, biologists today complement reductionism with **systems biology**, the exploration of a biological system by analyzing the interactions among its parts. In this context, a single leaf cell can be considered a system, as can a frog, an ant colony, or a desert ecosystem. By examining and modeling the dynamic behavior of an integrated network of components, systems biology enables us to pose new kinds of questions. For example, how do networks of molecular interactions in our bodies generate our 24-hour cycle of wakefulness and sleep? At a larger scale, how does a gradual increase in atmospheric carbon dioxide alter ecosystems and the entire biosphere? Systems biology can be used to study life at all levels.

Structure and Function

At each level of the biological hierarchy, we find a correlation of structure and function. Consider a leaf in Figure 1.3: Its thin, flat shape maximizes the capture of sunlight by chloroplasts. Because such correlations of structure and function are common in all forms of life, analyzing a biological structure gives us clues about what it does and how it works. Conversely,

knowing the function of something provides insight into its structure and organization.

Many examples from the animal kingdom show a correlation between structure and function. For example, the

hummingbird's anatomy allows the wings to rotate at the shoulder, so hummingbirds have the ability, unique among birds, to fly backward or hover in place. While hovering, the birds can extend their long,



slender beaks into flowers and feed on nectar. The elegant match of form and function in the structures of life is explained by natural selection, which we'll explore shortly.

The Cell: An Organism's Basic Unit of Structure and Function

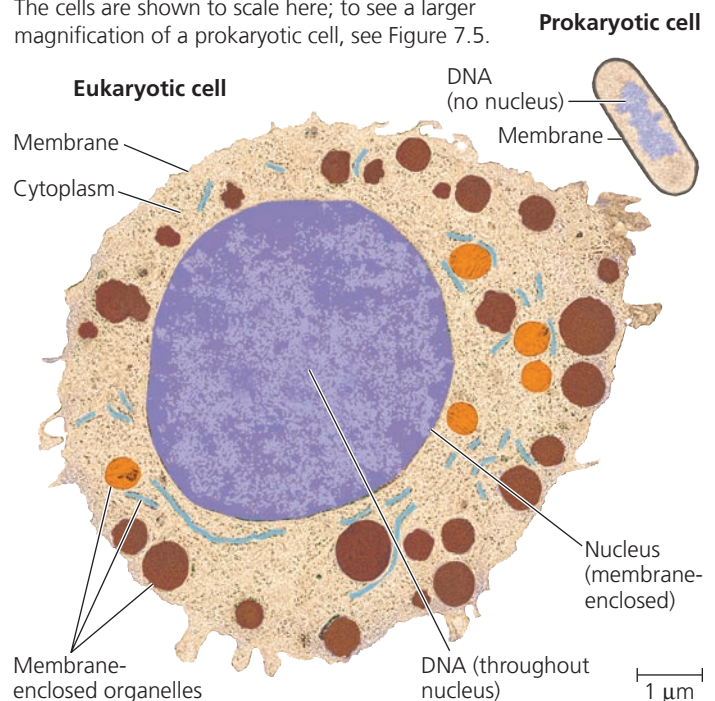
In life's structural hierarchy, the cell is the smallest unit of organization that can perform all activities required for life. The so-called Cell Theory was first developed in the 1800s, based on the observations of many scientists. The theory states that all living organisms are made of cells, which are the basic unit of life. In fact, the actions of organisms are all based on the functioning of cells. For instance, the movement of your eyes as you read this sentence results from the activities of muscle and nerve cells. Even a process that occurs on a global scale, such as the recycling of carbon atoms, is the product of cellular functions, including the photosynthetic activity of chloroplasts in leaf cells.

All cells share certain characteristics. For instance, every cell is enclosed by a membrane that regulates the passage of materials between the cell and its surroundings. Nevertheless, we distinguish two main forms of cells: prokaryotic and eukaryotic. The cells of two groups of single-celled microorganisms—bacteria (singular, *bacterium*) and archaea (singular, *archaeon*)—are prokaryotic. All other forms of life, including plants and animals, are composed of eukaryotic cells.

A **eukaryotic cell** contains membrane-enclosed organelles (Figure 1.4). Some organelles, such as the DNA-containing nucleus, are found in the cells of all eukaryotes; other organelles

▼ Figure 1.4 Contrasting eukaryotic and prokaryotic cells in size and complexity.

The cells are shown to scale here; to see a larger magnification of a prokaryotic cell, see Figure 7.5.



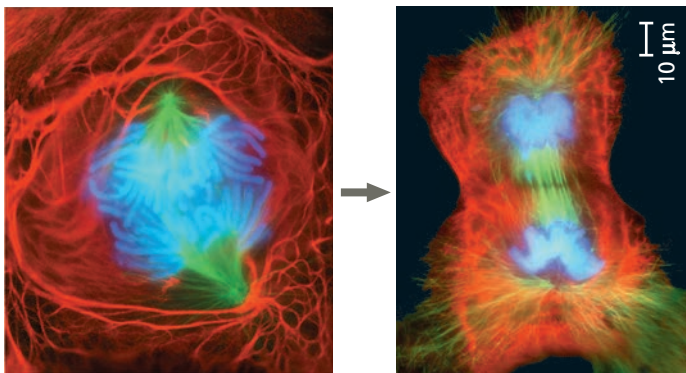
VISUAL SKILLS ► Measure the scale bar and use its length to estimate the length of the prokaryotic cell and the longest dimension of the eukaryotic cell.

are specific to particular cell types. For example, the chloroplast in Figure 1.3 is an organelle found only in eukaryotic cells that carry out photosynthesis. In contrast to eukaryotic cells, a **prokaryotic cell** lacks a nucleus or other membrane-enclosed organelles. Furthermore, prokaryotic cells are generally smaller than eukaryotic cells, as shown in Figure 1.4.

Theme: Life's Processes Involve the Expression and Transmission of Genetic Information

INFORMATION Within cells, structures called chromosomes contain genetic material in the form of **DNA (deoxyribonucleic acid)**. In cells that are preparing to divide, the chromosomes may be made visible using a dye that appears blue when bound to the DNA (**Figure 1.5**).

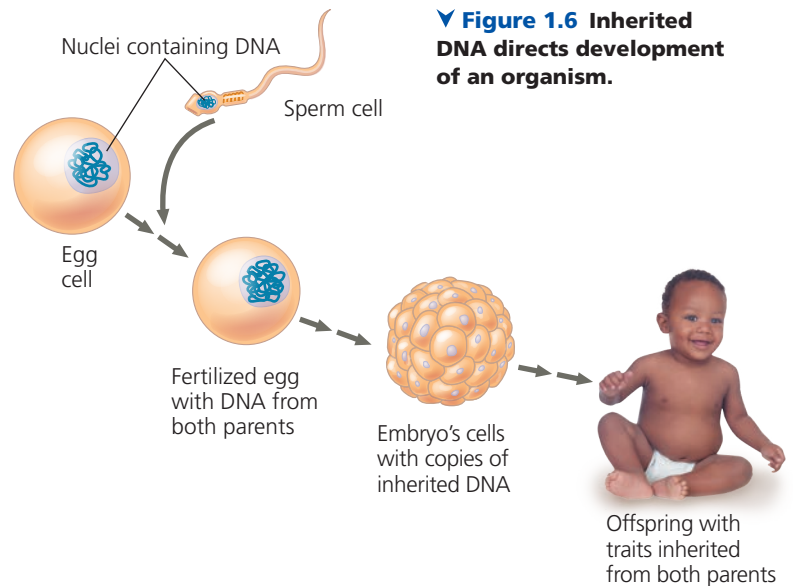
▼ **Figure 1.5** A lung cell from a newt divides into two smaller cells that will grow and divide again.



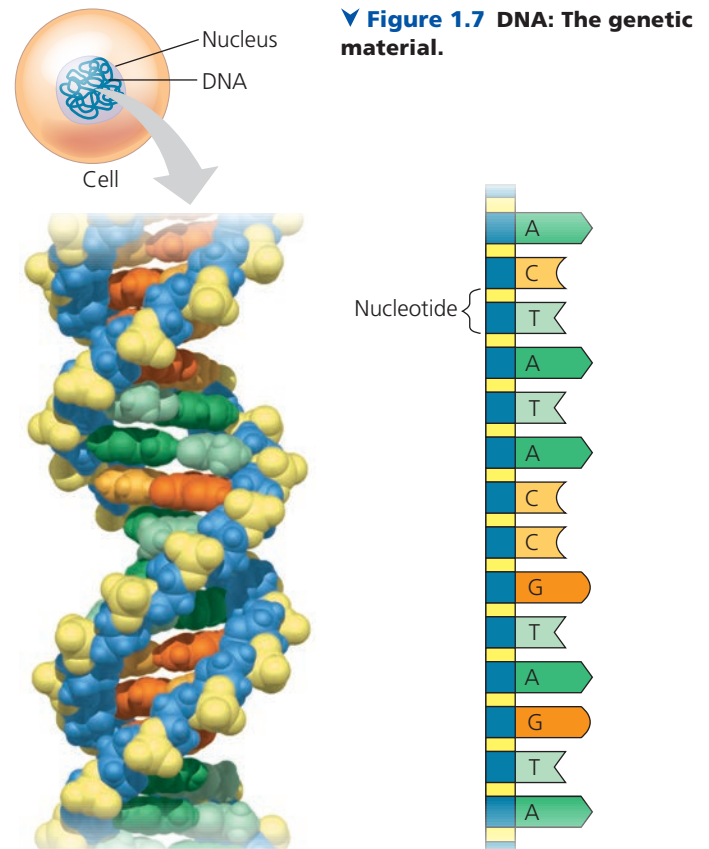
DNA, the Genetic Material

Before a cell divides, the DNA is first replicated, or copied, and each of the two cellular offspring inherits a complete set of chromosomes, identical to that of the parent cell. Each chromosome contains one very long DNA molecule with hundreds or thousands of **genes**, each a section of the DNA of the chromosome. Transmitted from parents to offspring, genes are the units of inheritance. They encode the information necessary to build all of the molecules synthesized within a cell, which in turn establish that cell's identity and function. You began as a single cell stocked with DNA inherited from your parents. The replication of that DNA prior to each cell division transmitted copies of the DNA to what eventually became the trillions of cells of your body. As the cells grew and divided, the genetic information encoded by the DNA directed your development (**Figure 1.6**).

The molecular structure of DNA accounts for its ability to store information. A DNA molecule is made up of two long chains, called strands, arranged in a double helix. Each chain is made up of four kinds of chemical building blocks called nucleotides, abbreviated A, T, C, and G (**Figure 1.7**). Specific sequences of these four nucleotides encode the information



▼ **Figure 1.6** Inherited DNA directs development of an organism.



▼ **Figure 1.7** DNA: The genetic material.

(a) DNA double helix. This model shows the atoms in a segment of DNA. Made up of two long chains (strands) of building blocks called nucleotides, a DNA molecule takes the three-dimensional form of a double helix.

(b) Single strand of DNA. These geometric shapes and letters are simple symbols for the nucleotides in a small section of one strand of a DNA molecule. Genetic information is encoded in specific sequences of the four types of nucleotides. Their names are abbreviated A, T, C, and G.



Animation: Heritable Information: DNA

in genes. The way DNA encodes information is analogous to how we arrange the letters of the alphabet into words and phrases with specific meanings. The word *rat*, for example, evokes a rodent; the words *tar* and *art*, which contain the same letters, mean very different things. We can think of nucleotides as a four-letter alphabet.

For many genes, the sequence provides the blueprint for making a protein. For instance, a given bacterial gene may specify a particular protein (an enzyme) required to break down a certain sugar molecule, while a human gene may denote a different protein (an antibody) that helps fight off infection. Overall, proteins are major players in building and maintaining the cell and carrying out its activities.

Protein-encoding genes control protein production indirectly, using a related molecule called RNA as an intermediary (**Figure 1.8**). The sequence of nucleotides along a gene is transcribed into mRNA, which is then translated into a linked series of protein building blocks called amino acids. Once completed, the amino acid chain forms a specific protein with a unique shape and function. The entire process by which the information in a gene directs the manufacture of a cellular product is called **gene expression**.

In carrying out gene expression, all forms of life employ essentially the same genetic code: A particular sequence of nucleotides says the same thing in one organism as it does in another. Differences between organisms reflect differences between their nucleotide sequences rather than between their genetic codes. This universality of the genetic code is a strong piece of evidence that all life is related. Comparing the sequences in several species for a gene that codes for a particular protein can provide valuable information both about the protein and about the relationship of the species to each other.

The mRNA molecule in Figure 1.8 is translated into a protein, but other cellular RNAs function differently. For example, we have known for decades that some types of RNA are actually components of the cellular machinery that manufactures proteins. Recently, scientists have discovered whole new classes of RNA that play other roles in the cell, such as regulating the functioning of protein-coding genes. Genes specify all of these RNAs as well, and their production is also referred to as gene expression. By carrying the instructions for making proteins and RNAs and by replicating with each cell division, DNA ensures faithful inheritance of genetic information from generation to generation.

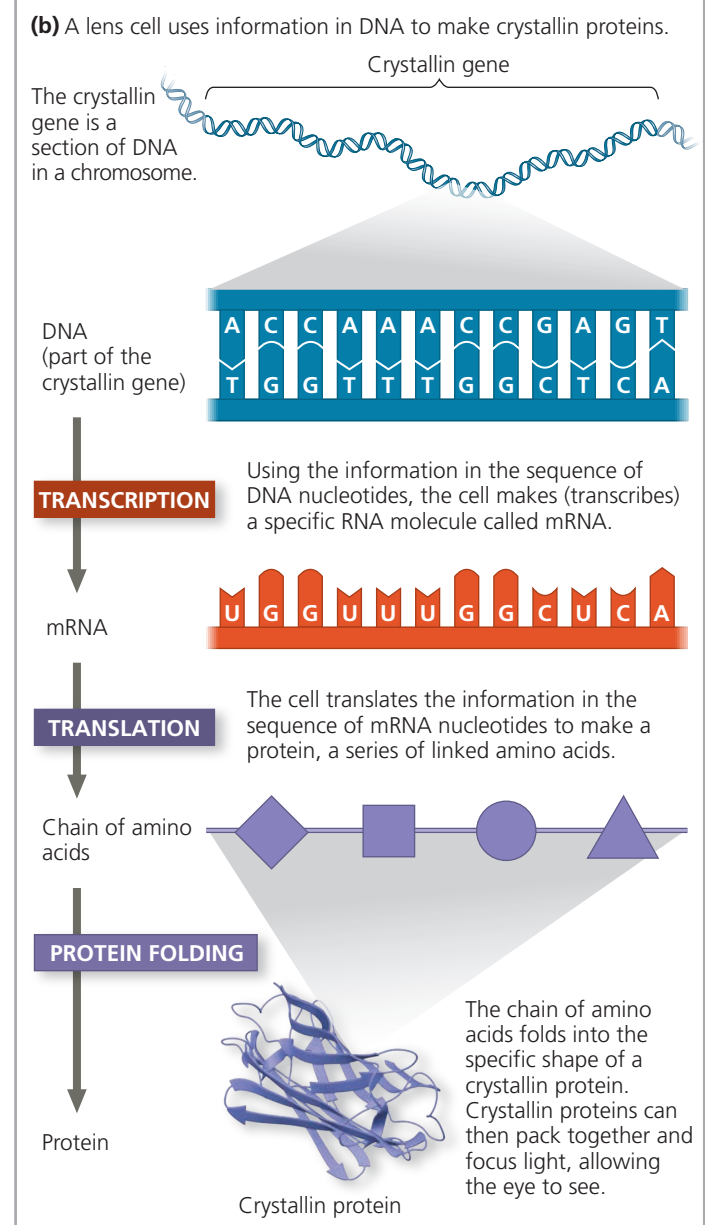
Genomics: Large-Scale Analysis of DNA Sequences

The entire “library” of genetic instructions that an organism inherits is called its **genome**. A typical human cell has two similar sets of chromosomes, and each set has approximately 3 billion nucleotide pairs of DNA. If the one-letter abbreviations for the nucleotides of a set were written in letters the

▼ **Figure 1.8 Gene expression: Cells use information encoded in a gene to synthesize a functional protein.**



- (a) The lens of the eye (behind the pupil) is able to focus light because lens cells are tightly packed with transparent proteins called crystallin. How do lens cells make crystallin proteins?
-



size of those you are now reading, the genomic text would fill about 700 biology textbooks.

Since the early 1990s, the pace at which researchers can determine the sequence of a genome has accelerated at an astounding rate, enabled by a revolution in technology. The genome sequence—the entire sequence of nucleotides for a representative member of a species—is now known for humans and many other animals, as well as numerous plants, fungi, bacteria, and archaea. To make sense of the deluge of data from genome-sequencing projects and the growing catalog of known gene functions, scientists are applying a systems biology approach at the cellular and molecular levels. Rather than investigating a single gene at a time, researchers study whole sets of genes (or other DNA) in one or more species—an approach called **genomics**. Likewise, the term **proteomics** refers to the study of sets of proteins and their properties. (The entire set of proteins expressed by a given cell, tissue, or organism is called a **proteome**).

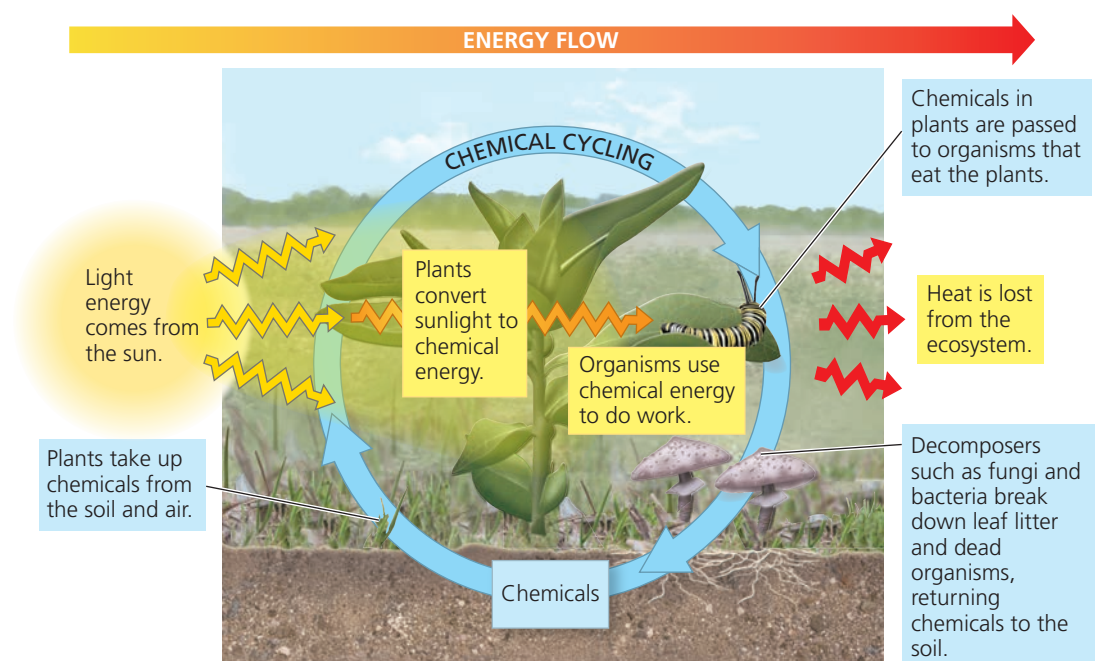
Three important research developments have made the genomic and proteomic approaches possible. One is “high-throughput” technology, tools that can analyze many biological samples very rapidly. The second major development is **bioinformatics**, the use of computational tools to store, organize, and analyze the huge volume of data that results from high-throughput methods. The third development is the formation of interdisciplinary research teams—groups of diverse specialists that may include computer scientists, mathematicians, engineers, chemists, physicists, and, of course, biologists from a variety of fields. Researchers in such teams aim to learn how the activities of all the proteins and RNAs encoded by the DNA are coordinated in cells and in whole organisms.

Theme: Life Requires the Transfer and Transformation of Energy and Matter

ENERGY AND MATTER A fundamental characteristic of living organisms is their use of energy to carry out life’s activities. Moving, growing, reproducing, and the various cellular activities of life are work, and work requires energy. The input of energy, primarily from the sun, and the transformation of energy from one form to another make life possible (**Figure 1.9**). When a plant’s leaves absorb sunlight, molecules within the leaves convert the energy of sunlight to the chemical energy of food, such as sugars, in the process of photosynthesis. The chemical energy in the food molecules is then passed along by plants and other photosynthetic organisms (**producers**) to consumers. **Consumers** are organisms, such as animals, that feed on other organisms or their remains.

When an organism uses chemical energy to perform work, such as muscle contraction or cell division, some of that energy is lost to the surroundings as heat. As a result, energy *flows through* an ecosystem in one direction, usually entering as light and exiting as heat. In contrast, chemicals *cycle within* an ecosystem, where they are used and then recycled (see Figure 1.9). Chemicals that a plant absorbs from the air or soil may be incorporated into the plant’s body and then passed to an animal that eats the plant. Eventually, these chemicals will be returned to the environment by decomposers such as bacteria and fungi that break down waste products, leaf litter, and the bodies of dead organisms. The chemicals are then available to be taken up by plants again, thereby completing the cycle.

➤ **Figure 1.9 Energy flow and chemical cycling.** There is a one-way flow of energy in an ecosystem: During photosynthesis, plants convert energy from sunlight to chemical energy (stored in food molecules such as sugars), which is used by plants and other organisms to do work and is eventually lost from the ecosystem as heat. In contrast, chemicals cycle between organisms and the physical environment.



Theme: From Molecules to Ecosystems, Interactions Are Important in Biological Systems

INTERACTIONS At any level of the biological hierarchy, interactions between the components of the system ensure smooth integration of all the parts, such that they function as a whole. This holds true equally well for molecules in a cell and the components of an ecosystem; we'll discuss both as examples.

Molecules: Interactions Within Organisms

At lower levels of organization, the interactions between components that make up living organisms—organs, tissues, cells, and molecules—are crucial to their smooth operation. Consider the regulation of blood sugar levels, for instance. Cells in the body must match the supply of fuel (sugar) to demand, regulating the opposing processes of sugar breakdown and storage. The key is the ability of many biological processes to self-regulate by a mechanism called feedback.

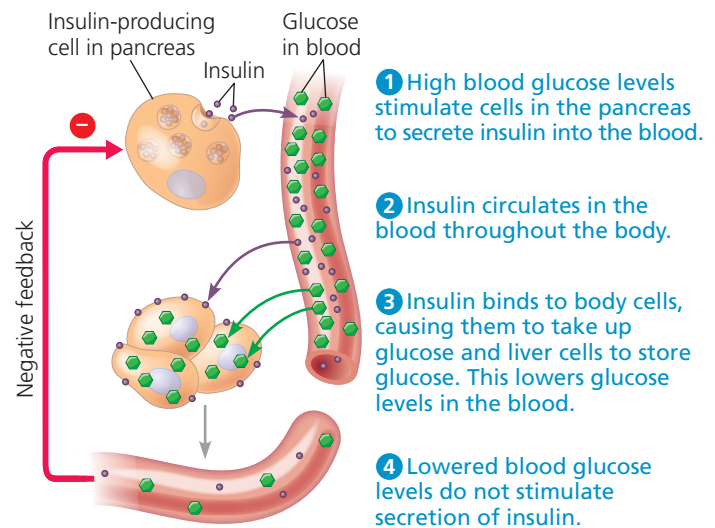
In **feedback regulation**, the output or product of a process regulates that very process. The most common form of regulation in living systems is *negative feedback*, a loop in which the response reduces the initial stimulus. As seen in the example of insulin signaling (**Figure 1.10**), after a meal the level of the sugar glucose in your blood rises, which stimulates cells of the pancreas to secrete insulin. Insulin, in turn, causes body cells to take up glucose and liver cells to store it, thus decreasing blood glucose levels. This eliminates the stimulus for insulin secretion, shutting off the pathway. Thus, the output of the process negatively regulates that process.

Though less common than processes regulated by negative feedback, there are also many biological processes regulated by *positive feedback*, in which an end product *speeds up* its own production. The clotting of your blood in response to injury is an example. When a blood vessel is damaged, structures in the blood called platelets begin to aggregate at the site. Positive feedback occurs as chemicals released by the platelets attract *more* platelets. The platelet pileup then initiates a complex process that seals the wound with a clot.

Ecosystems: An Organism's Interactions with Other Organisms and the Physical Environment

At the ecosystem level, every organism interacts with other organisms. For instance, an acacia tree interacts with soil microorganisms associated

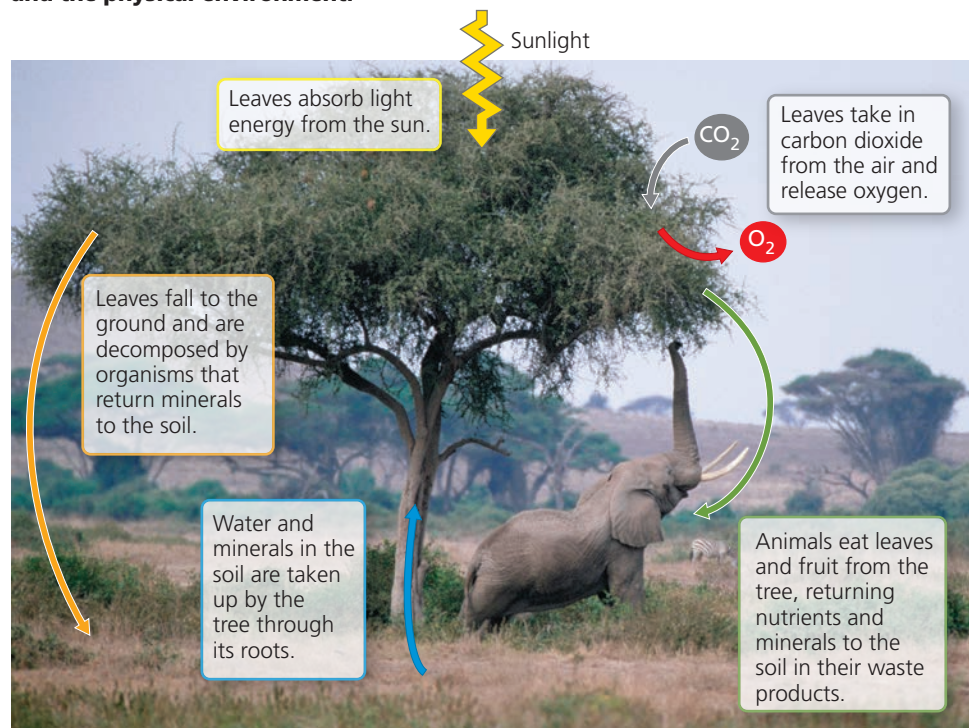
▼ **Figure 1.10 Feedback regulation.** The human body regulates use and storage of glucose, a major cellular fuel. This figure shows negative feedback: The response to insulin reduces the initial stimulus.



VISUAL SKILLS ► In this example, what is the response to insulin? What is the initial stimulus that is reduced by the response?

with its roots, insects that live on it, and animals that eat its leaves and fruit (**Figure 1.11**). Interactions between organisms include those that are mutually beneficial (as when “cleaner fish” eat small parasites on a turtle), and those in which one species benefits and the other is harmed (as when a lion kills and eats a zebra). In some interactions between species, both are harmed—for example, when two plants compete for a soil resource that is in short supply.

▼ **Figure 1.11 Interactions of an African acacia tree with other organisms and the physical environment.**



Interactions among organisms help regulate the functioning of the ecosystem as a whole.

Each organism also interacts continuously with physical factors in its environment. The leaves of a tree, for example, absorb light from the sun, take in carbon dioxide from the air, and release oxygen to the air (see Figure 1.11). The environment is also affected by organisms. For instance, in addition to taking up water and minerals from the soil, the roots of a plant break up rocks as they grow, contributing to the formation of soil. On a global scale, plants and other photosynthetic organisms have generated all the oxygen in the atmosphere.

Like other organisms, we humans interact with our environment. Our interactions sometimes have dire consequences: For example, over the past 150 years, humans have greatly increased the burning of fossil fuels (coal, oil, and gas). This practice releases large amounts of carbon dioxide (CO₂) and other gases into the atmosphere, causing heat to be trapped close to the Earth's surface (see Figure 56.29). Scientists calculate that the CO₂ that human activities have added to the atmosphere has increased the average temperature of the planet by about 1°C since 1900. At the current rates that CO₂ and other gases are being added to the atmosphere, global models predict an additional rise of at least 3°C before the end of this century.

This ongoing global warming is a major aspect of **climate change**, a directional change to the global climate that lasts for three decades or more (as opposed to short-term changes in the weather). But global warming is not the only way the climate is changing: Wind and precipitation patterns are also shifting, and extreme weather events such as storms and droughts are occurring more often. Climate change has already affected organisms and their habitats all over the planet. For example, polar bears have lost much of the ice platform from which they hunt, leading to food shortages and increased mortality rates. As habitats deteriorate, hundreds of plant and animal species are shifting their ranges to more suitable locations—but for some, there is insufficient suitable habitat, or they may not be able to migrate quickly enough. As a result, the populations of many species are shrinking in size or even disappearing (Figure 1.12). This

➤ **Figure 1.12 Threatened by global warming.** A warmer environment causes lizards in the genus *Sceloporus* to spend more time in refuges from the heat, reducing time for foraging. Their food intake drops, decreasing reproductive success. Surveys show that 12% of the 200 populations in Mexico have disappeared since 1975. For more examples of climate change affecting life on Earth, see Make Connections Figure 56.30.



trend can result in extinction, the permanent loss of a species. As we'll discuss in greater detail in Concept 56.4, the consequences of these changes for humans and other organisms may be profound.

Having considered four of the unifying themes (organization, information, energy and matter, and interactions), let's now turn to evolution. There is consensus among biologists that evolution is the core theme of biology, and it is discussed in detail in the next section.

CONCEPT CHECK 1.1

1. Starting with the molecular level in Figure 1.3, write a sentence that includes components from the previous (lower) level of biological organization, for example: "A molecule consists of *atoms* bonded together." Continue with organelles, moving up the biological hierarchy.
2. Identify the theme or themes exemplified by (a) the sharp quills of a porcupine, (b) the development of a multicellular organism from a single fertilized egg, and (c) a hummingbird using sugar to power its flight.
3. **WHAT IF? >** For each theme discussed in this section, give an example not mentioned in the text.

For suggested answers, see Appendix A.

CONCEPT 1.2

The Core Theme: Evolution accounts for the unity and diversity of life

EVOLUTION Evolution is the one idea that makes logical sense of everything we know about living organisms. As the fossil record clearly shows, life has been evolving on Earth for billions of years, resulting in a vast diversity of past and present organisms. But along with the diversity there is also unity, in the form of shared features. For example, while sea horses, jackrabbits, hummingbirds, and giraffes all look very different, their skeletons are organized in the same basic way.

The scientific explanation for the unity and diversity of organisms—as well as for the adaptation of organisms to their particular environments—is **evolution**: the concept that the organisms living on Earth today are the modified descendants of common ancestors. As a result of descent with modification, two species share certain traits (unity) simply because they have descended from a common ancestor. Furthermore, we can account for differences between two species (diversity) with the idea that certain heritable changes occurred after the two species diverged from their common ancestor. An abundance of evidence of different types supports the occurrence of evolution and the theory that describes how it takes place, which we'll discuss in detail in Unit 4. To quote one of the founders of modern evolutionary theory, Theodosius Dobzhansky, "Nothing in biology makes sense except in the light of evolution." To understand Dobzhansky's statement, we need to discuss how biologists think about the vast diversity of life on the planet.

Classifying the Diversity of Life

Diversity is a hallmark of life. Biologists have so far identified and named about 1.8 million species of organisms. Each species is given a two-part name: The first part is the name of the genus (plural, *genera*) to which the species belongs, and the second part is unique to the species within the genus. (For example, *Homo sapiens* is the name of our species.)

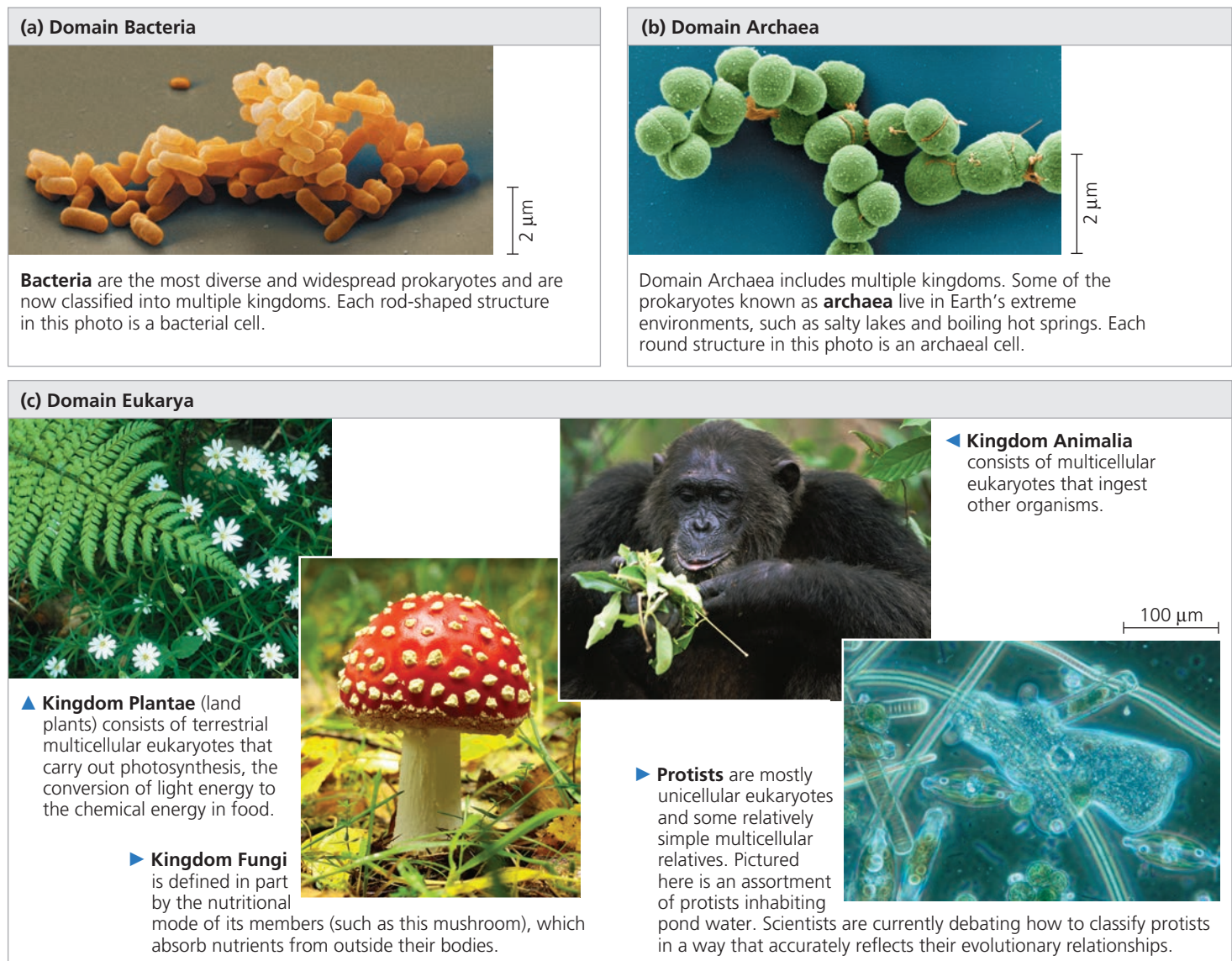
To date, known species include at least 100,000 species of fungi, 290,000 plant species, 57,000 vertebrate species (animals with backbones), and 1 million insect species (more than half of all known forms of life)—not to mention the myriad types of single-celled organisms. Researchers identify thousands of additional species each year. Estimates of the total number of species range from about 10 million to over 100 million. Whatever the actual number, the enormous variety of life gives biology a very broad scope. Biologists face a major challenge in attempting to make sense of this variety.

The Three Domains of Life

Historically, scientists have classified the diversity of life-forms into species and broader groupings by careful comparisons of structure, function, and other obvious features. In the last few decades, new methods of assessing species relationships, such as comparisons of DNA sequences, have led to a reevaluation of the classification of life. Although this reevaluation is ongoing, biologists currently divide all organisms into three groups called domains: Bacteria, Archaea, and Eukarya (**Figure 1.13**).

The organisms making up two of the three domains—**Bacteria** and **Archaea**—are prokaryotic. All the eukaryotes (organisms with eukaryotic cells) are in domain **Eukarya**. This domain includes four subgroups: kingdom Plantae, kingdom Fungi, kingdom Animalia, and the protists. The three kingdoms are distinguished partly by their modes of nutrition: Plants produce their own sugars and other food molecules by photosynthesis, fungi absorb nutrients in

▼ **Figure 1.13** The three domains of life.



dissolved form from their surroundings, and animals obtain food by eating and digesting other organisms. Animalia is, of course, the kingdom to which we belong.

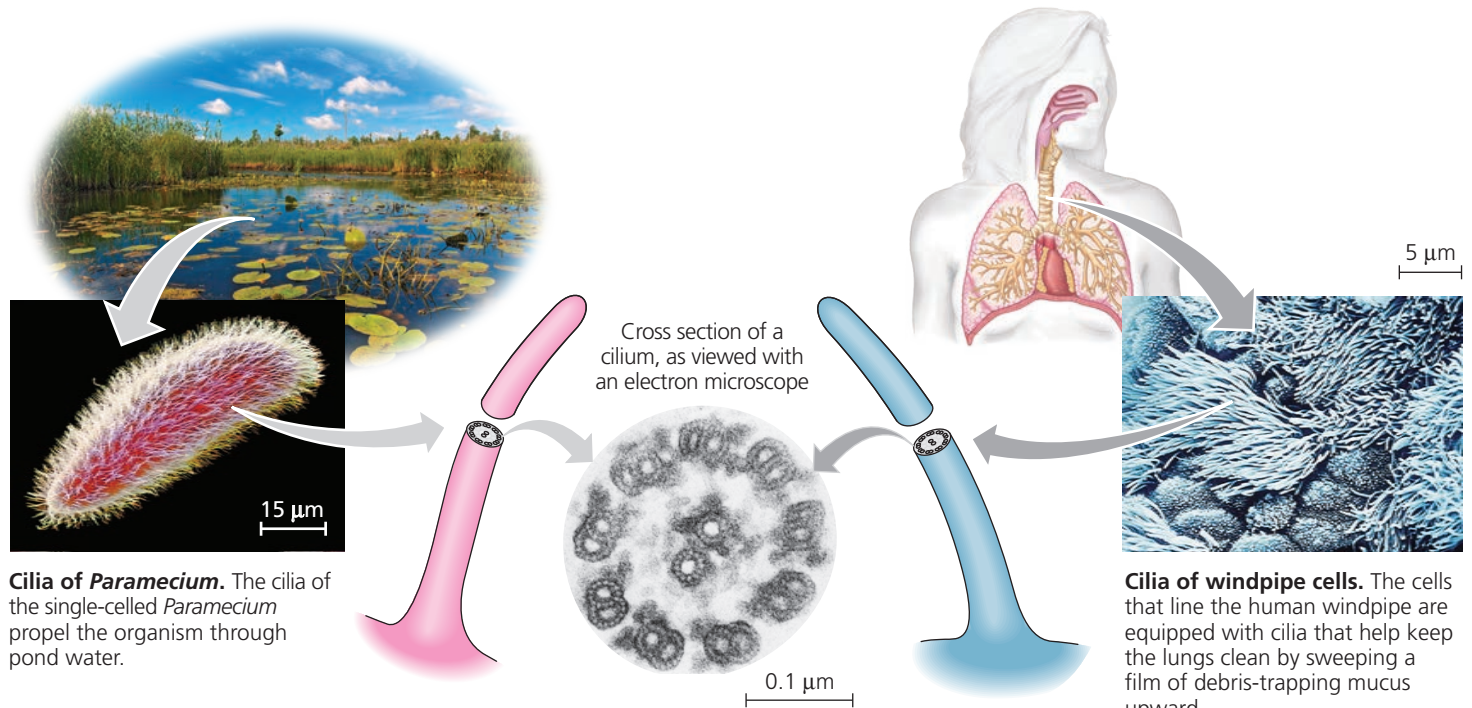
The most numerous and diverse eukaryotes are the protists, which are mostly single-celled organisms. Although protists were once placed in a single kingdom, they are now classified into several groups. One major reason for this change is the recent DNA evidence showing that some protists are less closely related to other protists than they are to plants, animals, or fungi.

Unity in the Diversity of Life

As diverse as life is, it also displays remarkable unity. Consider, for example, the similar skeletons of different animals and the universal genetic language of DNA (the genetic code), both mentioned earlier. In fact, similarities between organisms are evident at all levels of the biological hierarchy. For example, unity is obvious in many features of cell structure, even among distantly related organisms (Figure 1.14).

How can we account for life's dual nature of unity and diversity? The process of evolution, explained next, illuminates both the similarities and differences in the world of life. It also introduces another important dimension of biology: the passage of time. The history of life, as documented by

▼ **Figure 1.14 An example of unity underlying the diversity of life: the architecture of cilia in eukaryotes.** Cilia (singular, *cilium*) are extensions of cells that function in locomotion. They occur in eukaryotes as diverse as *Paramecium* (found in pond water) and humans. Even organisms so different share a common architecture for their cilia, which have an elaborate system of tubules that is striking in cross-sectional views.

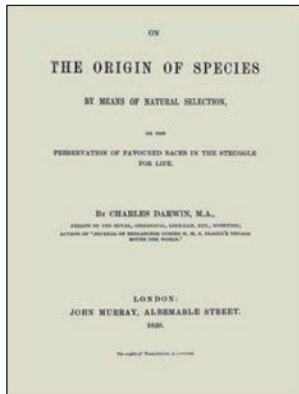


fossils and other evidence, is the saga of a changing Earth billions of years old, inhabited by an evolving cast of living forms (Figure 1.15).

▼ **Figure 1.15 Digging into the past.** Paleontologists carefully excavate the hind leg of a long-necked dinosaur (*Rapetosaurus krausei*) from rocks in Madagascar.



▼ **Figure 1.16 Charles Darwin as a young man.** His revolutionary book *On the Origin of Species* was first published in 1859.



 **ABC News Video: Exploring Evolution in the Solomon Islands**

Charles Darwin and the Theory of Natural Selection

An evolutionary view of life came into sharp focus in November 1859, when Charles Darwin published one of the most important and influential books ever written, *On the Origin of Species by Means of Natural Selection* (Figure 1.16). *The Origin of Species* articulated two main points. The first point was that contemporary species arose from a succession of ancestors that differed from them. Darwin called this process “descent with modification.” This insightful phrase captured the duality of life’s unity and diversity—unity in the kinship among species that descended from common ancestors and diversity in the modifications that evolved as species branched from their common ancestors (Figure 1.17). Darwin’s second main point was his proposal that “natural selection” is a primary cause of descent with modification.

Darwin developed his theory of natural selection from observations that by themselves were neither new nor profound. However, although others had described the pieces of the puzzle, it was Darwin who saw how they fit together. He started with the following three observations from nature: First, individuals in a population vary in their traits, many of which seem to be heritable (passed on from parents to offspring). Second, a population can produce far more offspring than can survive to produce offspring of their own. With more individuals than the environment is able to support, competition is inevitable. Third, species generally are suited to their environments—in other words, they are adapted to their circumstances. For instance, a common adaptation among birds that eat mostly hard seeds is an especially strong beak.

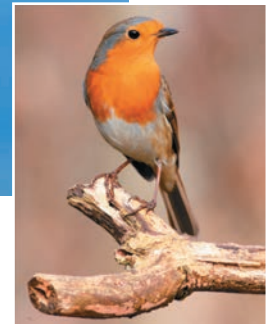
By making inferences from these three observations, Darwin arrived at his theory of evolution. He reasoned that individuals

▼ **Figure 1.17 Unity and diversity among birds.** These four birds are variations on a common body plan. For example, each has feathers, a beak, and wings. However, these common features are highly specialized for the birds’ diverse lifestyles.

▼ **Red-shouldered hawk**



▼ **American flamingo**



▲ **European robin**

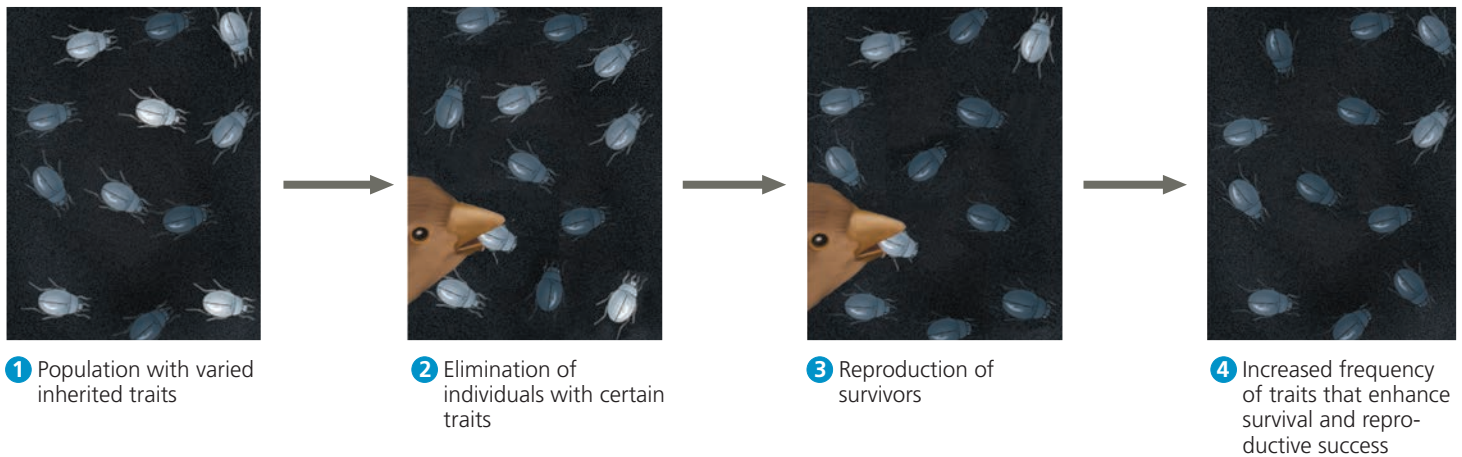


▲ **Gentoo penguin**

with inherited traits that are better suited to the local environment are more likely to survive and reproduce than less well-suited individuals. Over many generations, a higher and higher proportion of individuals in a population will have the advantageous traits. Evolution occurs as the unequal reproductive success of individuals ultimately leads to adaptation to their environment, as long as the environment remains the same.

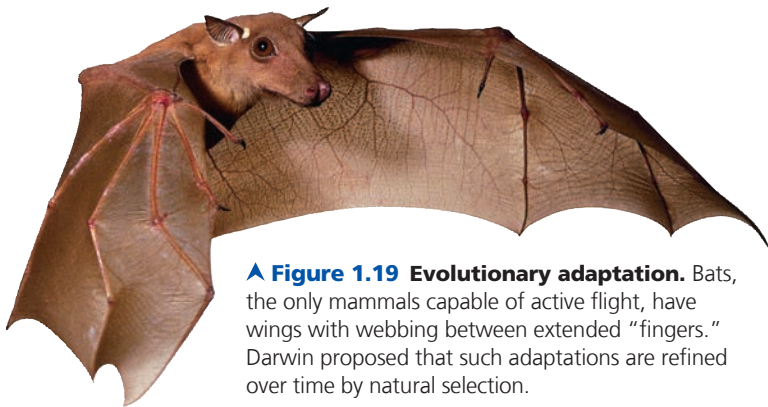
Darwin called this mechanism of evolutionary adaptation **natural selection** because the natural environment consistently “selects” for the propagation of certain traits among naturally occurring variant traits in the population. The example in Figure 1.18 illustrates the ability of natural selection to “edit” a population’s heritable variations in color. We see the products of natural selection in the exquisite adaptations of various organisms to the special circumstances of their way of life and their environment. The wings of the bat shown in Figure 1.19 are an excellent example of adaptation.

▼ **Figure 1.18 Natural selection.** This imaginary beetle population has colonized a locale where the soil has been blackened by a recent brush fire. Initially, the population varies extensively in the inherited coloration of the individuals, from very light gray to charcoal. For hungry birds that prey on the beetles, it is easiest to spot the beetles that are lightest in color.



DRAW IT ► Over time, the soil will gradually become lighter in color. Draw another step to show how the soil, when lightened to medium color, would affect natural selection. Write a caption for this new step. Then explain how the population would change over time as the soil becomes lighter.

 **HHMI Video: The Making of The Fittest: Natural Selection and Adaptation (Rock Pocket Mouse)**



▲ **Figure 1.19 Evolutionary adaptation.** Bats, the only mammals capable of active flight, have wings with webbing between extended “fingers.” Darwin proposed that such adaptations are refined over time by natural selection.

The Tree of Life

Take another look at the skeletal architecture of the bat’s wings in Figure 1.19. These wings are not like those of feathered birds; the bat is a mammal. The bat’s forelimbs, though adapted for flight, actually have all the same bones, joints, nerves, and blood vessels found in other limbs as diverse as the human arm, the foreleg of a horse, and the flipper of a whale. Indeed, all mammalian forelimbs are anatomical variations of a common architecture. According to the Darwinian concept of descent with modification, the shared anatomy of mammalian limbs reflects inheritance of the limb structure from a common ancestor—the “prototype” mammal from which all other mammals descended. The diversity of mammalian forelimbs results from modification by natural selection operating over millions of years in different environmental contexts. Fossils and other evidence corroborate anatomical unity in supporting this view of mammalian descent from a common ancestor.

Darwin proposed that natural selection, by its cumulative effects over long periods of time, could cause an ancestral species to give rise to two or more descendant species. This could occur, for example, if one population fragmented into several subpopulations isolated in different environments. In these separate arenas of natural selection, one species could gradually radiate into multiple species as the geographically isolated populations adapted over many generations to different sets of environmental factors.

The Galápagos finches are a famous example of the process of radiation of new species from a common ancestor. Darwin collected specimens of these birds during his 1835 visit to the remote Galápagos Islands, 900 kilometers (km) off the Pacific coast of South America. These relatively young volcanic islands are home to many species of plants and animals found nowhere else in the world, though many Galápagos organisms are clearly related to species on the South American mainland. The Galápagos finches are believed to have descended from an ancestral finch species that reached the archipelago from South America or the Caribbean. Over time, the Galápagos finches diversified from their ancestor as populations became adapted to different food sources on their particular islands. Years after Darwin collected the finches, researchers began to sort out their evolutionary relationships, first from anatomical and geographic data and more recently with the help of DNA sequence comparisons.

 **ABC News Video: Protecting the Galápagos Islands**

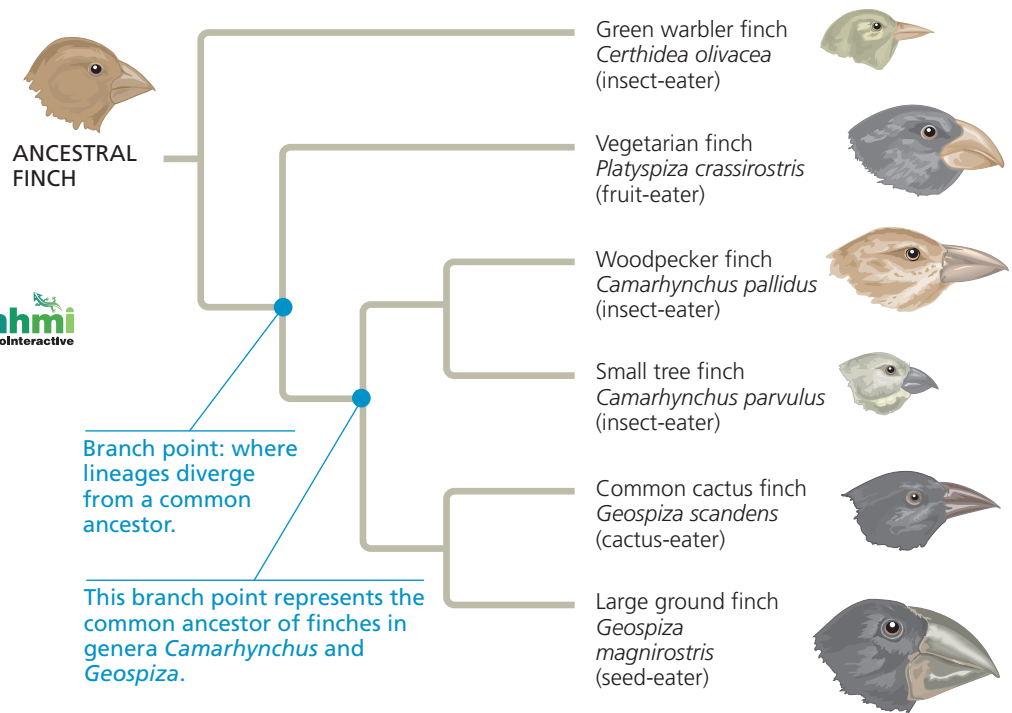
Biologists’ diagrams of evolutionary relationships generally take treelike forms, though the trees are often turned sideways

► **Figure 1.20** Descent with modification: adaptive radiation of finches on the Galápagos Islands.

This “tree” illustrates a current model for the evolution of finches on the Galápagos. Note the different beaks, which are adapted to different food sources on the different islands. For example, heavier, thicker beaks are better at cracking seeds, while the more slender beaks are better at grasping insects.

 **HHMI Video: The Origin of Species: The Beak of the Finch**

 **hhmi**
BioInteractive



as in **Figure 1.20**. Tree diagrams make sense: Just as an individual has a genealogy that can be diagrammed as a family tree, each species is one twig of a branching tree of life extending back in time through ancestral species more and more remote. Species that are very similar, such as the Galápagos finches, share a common ancestor. Through an ancestor that lived much farther back in time, finches are related to sparrows, hawks, penguins, and all other birds. Furthermore, finches and other birds are related to us through a common ancestor even more ancient. Trace life back far enough, and we reach the early prokaryotes that inhabited Earth over 3.5 billion years ago. We can recognize their vestiges in our own cells—in the universal genetic code, for example. Indeed, all of life is connected through its long evolutionary history.

CONCEPT CHECK 1.2

1. Explain why “editing” is a metaphor for how natural selection acts on a population’s heritable variation.
2. Referring to Figure 1.20, provide a possible explanation for how, over a very long time, the green warbler finch came to have a slender beak.
3. **DRAW IT** ► The three domains you learned about in Concept 1.2 can be represented in the tree of life as the three main branches, with three subbranches on the eukaryotic branch being the kingdoms Plantae, Fungi, and Animalia. What if fungi and animals are more closely related to each other than either of these kingdoms is to plants—as recent evidence strongly suggests? Draw a simple branching pattern that symbolizes the proposed relationship between these three eukaryotic kingdoms.

For suggested answers, see Appendix A.

CONCEPT 1.3

In studying nature, scientists make observations and form and test hypotheses

Science is a way of knowing—an approach to understanding the natural world. It developed out of our curiosity about ourselves, other life-forms, our planet, and the universe. The word *science* is derived from a Latin verb meaning “to know.” Striving to understand seems to be one of our basic urges.

At the heart of science is **inquiry**, a search for information and explanations of natural phenomena. There is no formula for successful scientific inquiry, no single scientific method that researchers must rigidly follow. As in all quests, science includes elements of challenge, adventure, and luck, along with careful planning, reasoning, creativity, patience, and the persistence to overcome setbacks. Such diverse elements of inquiry make science far less structured than most people realize. That said, it is possible to highlight certain characteristics that help to distinguish science from other ways of describing and explaining nature.

Scientists use a process of inquiry that includes making observations, forming logical, testable explanations (*hypotheses*), and testing them. The process is necessarily repetitive: In testing a hypothesis, more observations may inspire revision of the original hypothesis or formation of a new one, thus leading to further testing. In this way,

scientists circle closer and closer to their best estimation of the laws governing nature.

Exploration and Observation

Our innate curiosity often stimulates us to pose questions about the natural basis for the phenomena we observe in the world. For example, what causes the roots of a plant seedling to grow downward? In fine-tuning their questions, biologists rely heavily on the scientific literature, the published contributions of fellow scientists. By reading about and understanding past studies, scientists can build on the foundation of existing knowledge, focusing their investigations on observations that are original and on hypotheses that are consistent with previous findings. Identifying publications relevant to a new line of research is now easier than at any point in the past, thanks to indexed and searchable electronic databases.

In the course of their work, biologists make careful observations. In gathering information, they often use tools such as microscopes, precision thermometers, or high-speed cameras that extend their senses or facilitate careful measurement. Observations can reveal valuable information about the natural world. For example, a series of detailed observations have shaped our understanding of cell structure, and another set of observations is currently expanding our databases of genome sequences from diverse species and databases of genes whose expression is altered in various diseases.

Recorded observations are called **data**. Put another way, data are items of information on which scientific inquiry is based. The term *data* implies numbers to many people. But some data are *qualitative*, often in the form of recorded descriptions rather than numerical measurements. For example, Jane Goodall spent decades recording her observations of chimpanzee behavior during field research in a Tanzanian jungle (Figure 1.21). In her studies, Goodall also enriched the field of animal behavior with volumes of *quantitative* data, such as the frequency and duration of specific behaviors for different members of a group of chimpanzees in a variety of situations. Quantitative data are generally expressed as numerical measurements and often organized into tables and graphs. Scientists analyze their data using a type of mathematics called *statistics* to test whether their results are significant or merely due to random fluctuations. All results presented in this text have been shown to be statistically significant.

Collecting and analyzing observations can lead to important conclusions based on a type of logic called **inductive reasoning**. Through induction, we derive generalizations from a large number of specific observations. “The sun always rises in the east” is an example. And so is “All organisms are made of cells.” Careful observations and data analyses, along

Figure 1.21 Jane Goodall collecting qualitative data on chimpanzee behavior. Goodall recorded her observations in field notebooks, often with sketches of the animals’ behavior.



Interview with Jane Goodall: Living with chimpanzees

with generalizations reached by induction, are fundamental to our understanding of nature.

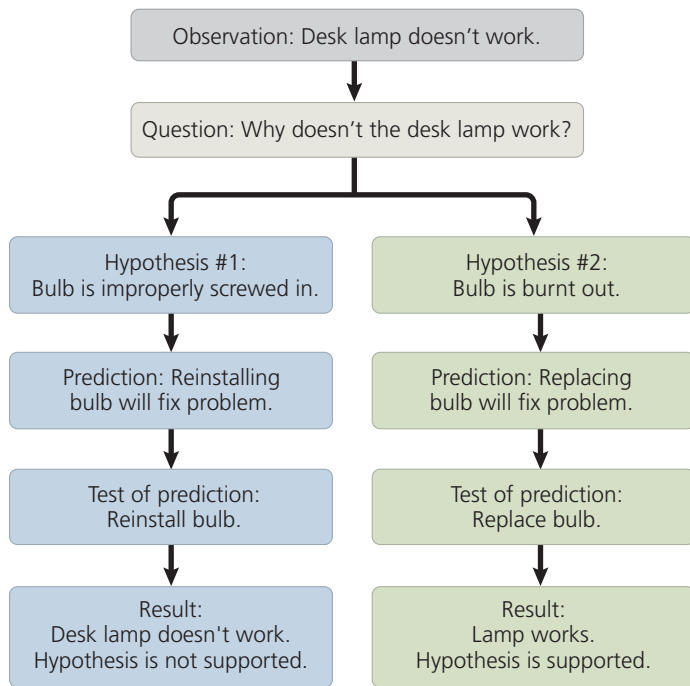
Forming and Testing Hypotheses

After carrying out preliminary observations and collecting and analyzing data, scientists begin to form tentative answers to their original questions and to test their hypothetical explanations—that is, their hypotheses. In science, a **hypothesis** is an explanation, based on observations and assumptions, that leads to a testable prediction. Said another way, a hypothesis is an explanation on trial. The hypothesis is usually a rational accounting for a set of observations, based on the available data and guided by inductive reasoning. A scientific hypothesis must lead to predictions that can be tested by making additional observations or by performing experiments. An **experiment** is a scientific test, carried out under controlled conditions.

We all make observations and develop questions and hypotheses in solving everyday problems. Let’s say, for example, that your desk lamp is plugged in and turned on but the bulb isn’t lit. That’s an observation. The question is obvious: Why doesn’t the lamp work? Two reasonable hypotheses based on your experience are that (1) the bulb is not screwed in properly or (2) the bulb is burnt out. Each of these alternative hypotheses leads to predictions you can test with experiments. For example, the improperly screwed-in

▼ **Figure 1.22 A simplified view of the scientific process.**

The idealized process sometimes called the “scientific method” is shown in this flow chart, which illustrates hypothesis testing for a desk lamp that doesn’t work.



bulb hypothesis predicts that carefully re-installing the bulb will fix the problem. **Figure 1.22** diagrams this informal inquiry. Figuring things out in this way by trial and error is a hypothesis-based approach.

Deductive Reasoning

A type of logic called deduction is also built into the use of hypotheses in science. While induction entails reasoning from a set of specific observations to reach a general conclusion, **deductive reasoning** involves logic that flows in the opposite direction, from the general to the specific. From general premises, we extrapolate to the specific results we should expect if the premises are true. In the scientific process, deductions usually take the form of predictions of results that will be found if a particular hypothesis (premise) is correct. We then test the hypothesis by carrying out experiments or observations to see whether or not the results are as predicted. This deductive testing takes the form of “If . . . then” logic. In the case of the desk lamp example: *If* the burnt-out bulb hypothesis is correct, *then* the lamp should work if you replace the bulb with a new one.

We can use the desk lamp example to illustrate two other key points about the use of hypotheses in science. First, one can always devise additional hypotheses to explain a set of observations. For instance, another hypothesis to explain our nonworking desk lamp is that the electrical socket is

broken. Although you could design an experiment to test this hypothesis, you can never test all possible hypotheses. Second, we can never *prove* that a hypothesis is true. Based on the experiments shown in Figure 1.22, the burnt-out bulb hypothesis is the most likely explanation, but testing supports that hypothesis *not* by proving that it is correct, but rather by failing to prove it incorrect. For example, even if replacing the bulb fixed the desk lamp, it might have been because there was a temporary power outage that just happened to end while the bulb was being changed.

Although a hypothesis can never be proved beyond the shadow of a doubt, testing it in various ways can significantly increase our confidence in its validity. Often, rounds of hypothesis formulation and testing lead to a scientific consensus—the shared conclusion of many scientists that a particular hypothesis explains the known data well and stands up to experimental testing.

Questions That Can and Cannot Be Addressed by Science

Scientific inquiry is a powerful way to learn about nature, but there are limitations to the kinds of questions it can answer. A scientific hypothesis must be *testable*; there must be some observation or experiment that could reveal if such an idea is likely to be true or false. The hypothesis that a burnt-out bulb is the sole reason the lamp doesn’t work would not be supported if replacing the bulb with a new one didn’t fix the lamp.

Not all hypotheses meet the criteria of science: You wouldn’t be able to test the hypothesis that invisible ghosts are fooling with your desk lamp! Because science only deals with natural, testable explanations for natural phenomena, it can neither support nor contradict the invisible ghost hypothesis, nor whether spirits or elves cause storms, rainbows, or illnesses. Such supernatural explanations are simply outside the bounds of science, as are religious matters, which are issues of personal faith. Science and religion are not mutually exclusive or contradictory; they are simply concerned with different issues.

The Flexibility of the Scientific Process

The desk lamp example of Figure 1.22 traces an idealized process of inquiry sometimes called the *scientific method*. However, very few scientific inquiries adhere rigidly to the sequence of steps that are typically used to describe this approach. For example, a scientist may start to design an experiment, but then backtrack after realizing that more preliminary observations are necessary. In other cases, observations remain too puzzling to prompt well-defined questions until further study provides a new context in which to view those observations. For example, scientists

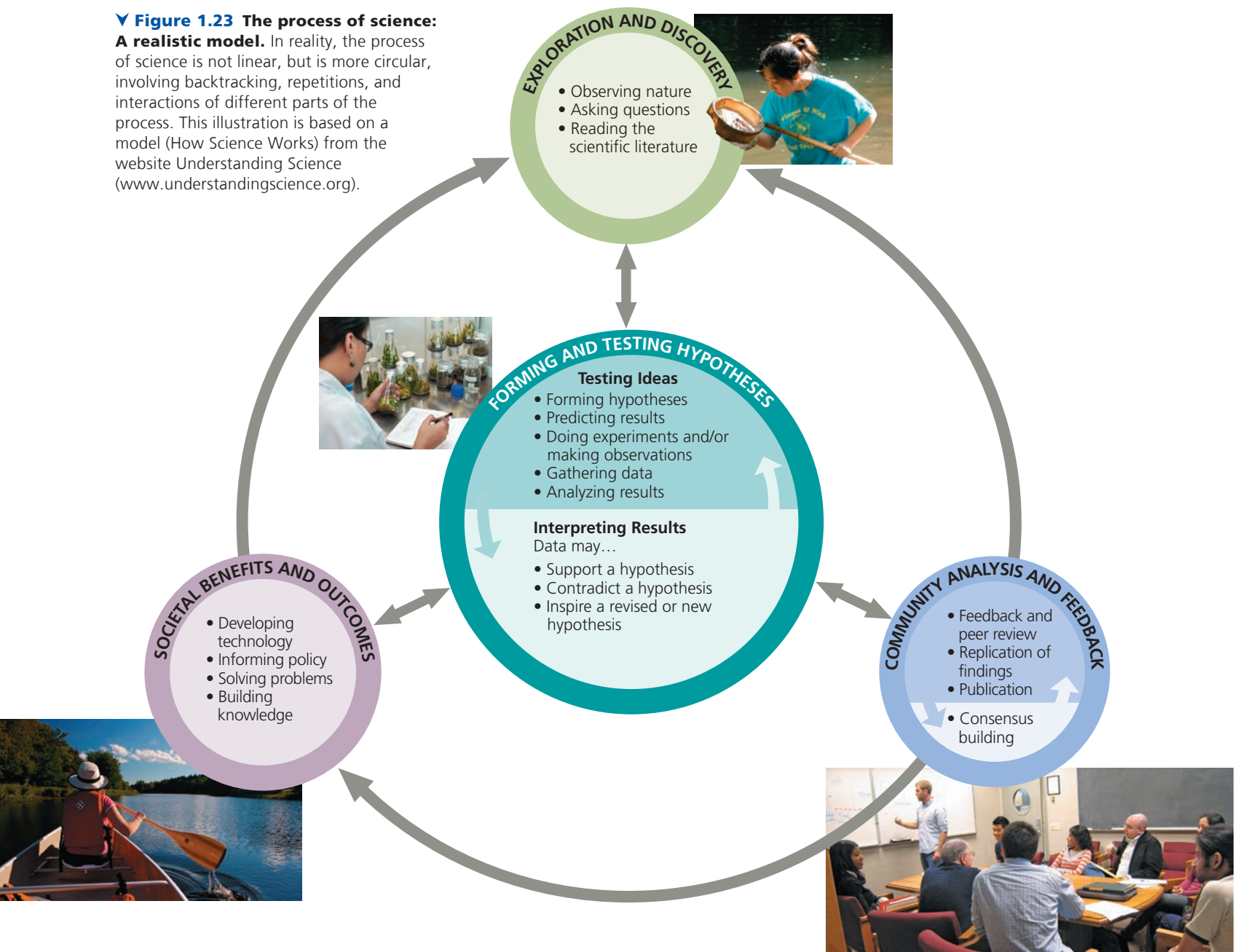
could not unravel the details of how genes encode proteins until *after* the discovery of the structure of DNA (an event that took place in 1953).

A more realistic model of the scientific process is shown in **Figure 1.23**. The focus of this model, shown in the central circle in the figure, is the forming and testing of hypotheses. This core set of activities is the reason that science does so well in explaining phenomena in the natural world. These activities, however, are shaped by exploration and discovery (the upper circle in Figure 1.23) and influenced by interactions with other scientists and with society more generally

(lower circles). For example, the community of scientists influences which hypotheses are tested, how test results are interpreted, and what value is placed on the findings. Similarly, societal needs—such as the push to cure cancer or understand the process of climate change—may help shape what research projects are funded and how extensively the results are discussed.

Now that we have highlighted the key features of scientific inquiry—making observations and forming and testing hypotheses—you should be able to recognize these features in a case study of actual scientific research.

▼ **Figure 1.23 The process of science: A realistic model.** In reality, the process of science is not linear, but is more circular, involving backtracking, repetitions, and interactions of different parts of the process. This illustration is based on a model (How Science Works) from the website Understanding Science (www.understandingscience.org).



A Case Study in Scientific Inquiry: Investigating Coat Coloration in Mouse Populations

Our case study begins with a set of observations and inductive generalizations. Color patterns of animals vary widely in nature, sometimes even among members of the same species. What accounts for such variation? As you may recall, the two mice depicted at the beginning of this chapter are members of the same species (*Peromyscus polionotus*), but they have different color patterns and reside in different environments. The beach mouse lives along the Florida seashore, a habitat of brilliant white sand dunes with sparse clumps of beach grass. The inland mouse lives on darker, more fertile soil farther inland (Figure 1.24). Even a brief glance at the photographs in Figure 1.24 reveals a striking match of mouse coloration to its habitat. The natural predators of these mice, including hawks, owls, foxes, and coyotes, are all visual hunters (they use their eyes to look for prey). It was logical, therefore, for Francis Bertody Sumner, a naturalist studying populations of these mice in the 1920s, to form the hypothesis that their coloration patterns had evolved as adaptations that camouflage the mice in their native environments, protecting them from predation.

As obvious as the camouflage hypothesis may seem, it still required testing. In 2010, biologist Hopi Hoekstra of Harvard University and a group of her students headed to Florida to test the prediction that mice with coloration that did not match their habitat would be preyed on more heavily than the native, well-matched mice. Figure 1.25 summarizes this field experiment.

The researchers built hundreds of models of mice and spray-painted them to resemble either beach or inland mice, so that

the models differed only in their color patterns. The researchers placed equal numbers of these model mice randomly in both habitats and left them overnight. The mouse models resembling the native mice in the habitat were the *control* group (for instance, light-colored mouse models in the beach habitat), while the mouse models with the non-native coloration were the *experimental* group (for example, darker models in the beach habitat). The following morning, the team counted and recorded signs of predation events, which ranged from bites and gouge marks on some models to the outright disappearance of others. Judging by the shape of the predators' bites and the tracks surrounding the experimental sites, the predators appeared to be split fairly evenly between mammals (such as foxes and coyotes) and birds (such as owls, herons, and hawks).

For each environment, the researchers then calculated the percentage of predation events that targeted camouflaged models. The results were clear-cut: Camouflaged models showed much lower predation rates than those lacking camouflage in both the beach habitat (where light mice were less vulnerable) and the inland habitat (where dark mice were less vulnerable). The data thus fit the key prediction of the camouflage hypothesis.

Experimental Variables and Controls

In carrying out an experiment, a researcher often manipulates one factor in a system and observes the effects of this change. The mouse camouflage experiment described in Figure 1.25 is an example of a **controlled experiment**, one that is designed to compare an experimental group (the non-camouflaged mice models, in this case) with a control group (the camouflaged models). Both the factor that is manipulated and the factor that is subsequently measured are types of experimental **variables**—a feature or quantity that varies in

✓ **Figure 1.24** Different coloration in beach and inland populations of *Peromyscus polionotus*.



Beach mice living on sparsely vegetated sand dunes along the coast have light tan, dappled fur on their backs that allows them to blend into their surroundings, providing camouflage.

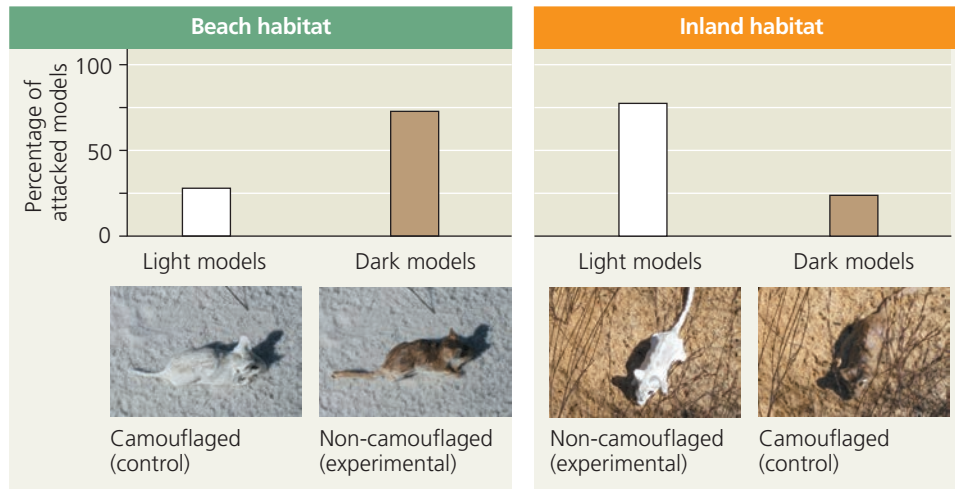
Members of the same species living about 30 km inland have dark fur on their backs, camouflaging them against the dark ground of their habitat.

▼ Figure 1.25

Inquiry Does camouflage affect predation rates on two populations of mice?

Experiment Hopi Hoekstra and colleagues tested the hypothesis that coat coloration provides camouflage that protects beach and inland populations of *Peromyscus polionotus* mice from predation in their habitats. The researchers spray-painted mouse models with light or dark color patterns that matched those of the beach and inland mice and placed models with each of the patterns in both habitats. The next morning, they counted damaged or missing models.

Results For each habitat, the researchers calculated the percentage of attacked models that were camouflaged or non-camouflaged. In both habitats, the models whose pattern did not match their surroundings suffered much higher “predation” than did the camouflaged models.



Conclusion The results are consistent with the researchers’ prediction: that mouse models with camouflage coloration would be preyed on less often than non-camouflaged mouse models. Thus, the experiment supports the camouflage hypothesis.

Data from S. N. Vignieri, J. G. Larson, and H. E. Hoekstra, The selective advantage of crypsis in mice, *Evolution* 64:2153–2158 (2010).

INTERPRET THE DATA ▶ The bars indicate the percentage of the attacked models that were either light or dark. Assume 100 mouse models were attacked in each habitat. For the beach habitat, how many were light models? Dark models? Answer the same questions for the inland habitat.

an experiment. In our example, the color of the mouse model was the **independent variable**—the factor being manipulated by the researchers. The **dependent variable** is the factor being measured that is predicted to be affected by the independent variable; in this case, the researchers measured the predation rate in response to variation in color of the mouse model. Ideally, the experimental and control groups differ in only one independent variable—in the mouse experiment, color.

Without the control group, the researchers would not have been able to rule out other factors as causes of the more frequent attacks on the non-camouflaged mice—such as different numbers of predators or different temperatures in the different test areas. The clever experimental design left coloration as the only factor that could account for the low predation rate on models camouflaged with respect to the surrounding environment.

A common misconception is that the term *controlled experiment* means that scientists control all features of the experimental environment. But that’s impossible in field research and can be very difficult even in highly regulated laboratory environments. Researchers usually “control” unwanted variables not by *eliminating* them through environmental regulation, but by *canceling out* their effects by using control groups.

Animation: Introduction to Experimental Design

Theories in Science

“It’s just a theory!” Our everyday use of the term *theory* often implies an untested speculation. But the term *theory* has a different meaning in science. What is a scientific theory, and how is it different from a hypothesis or from mere speculation?

First, a scientific **theory** is much broader in scope than a hypothesis. *This* is a hypothesis: “Coat coloration well-matched to their habitat is an adaptation that protects mice from predators.” But *this* is a theory: “Evolutionary adaptations arise by natural selection.” This theory proposes that natural selection is the evolutionary mechanism that accounts for an enormous variety of adaptations, of which coat color in mice is but one example.

Second, a theory is general enough to spin off many new, testable hypotheses. For example, the theory of natural selection motivated two researchers at Princeton University, Peter and Rosemary Grant, to test the specific hypothesis that the beaks of Galápagos finches evolve in response to changes in the types of available food. (Their results supported their hypothesis; see the introduction to Chapter 23.)

And third, compared to any one hypothesis, a theory is generally supported by a much greater body of evidence. The theory of natural selection has been supported by a vast quantity of evidence, with more being found every day, and has not been contradicted by any scientific data. Those theories that become widely adopted in science (such as the theory of natural selection and the theory of gravity) explain a great diversity of observations and are supported by a vast accumulation of evidence.

In spite of the body of evidence supporting a widely accepted theory, scientists will sometimes modify or even reject theories when new research produces results that don't fit. For example, biologists once lumped bacteria and archaea together as a kingdom of prokaryotes. When new methods for comparing cells and molecules could be used to test such relationships, the evidence led scientists to reject the theory that bacteria and archaea are members of the same kingdom. If there is "truth" in science, it is at best conditional, based on the weight of available evidence.

CONCEPT CHECK 1.3

1. What qualitative observation led to the quantitative study in Figure 1.25?
2. Contrast inductive reasoning with deductive reasoning.
3. Why is natural selection called a theory?
4. **WHAT IF? >** In the deserts of New Mexico, the soils are mostly sandy, with occasional regions of black rock derived from lava flows that occurred about 1,000 years ago. Mice are found in both sandy and rocky areas, and owls are known predators. What might you expect about coat color in these two mouse populations? Explain. How would you use this ecosystem to further test the camouflage hypothesis?

For suggested answers, see Appendix A.

CONCEPT 1.4

Science benefits from a cooperative approach and diverse viewpoints

Movies and cartoons sometimes portray scientists as loners in white lab coats, working in isolated labs. In reality, science is an intensely social activity. Most scientists work in teams, which often include both graduate and undergraduate students. And to succeed in science, it helps to be a good communicator. Research results have no impact until shared with a community of peers through seminars, publications, and websites. And, in fact, research papers aren't published until they are vetted by colleagues in what is called the "peer review" process. The examples of scientific inquiry described in this book, for instance, have all been published in peer-reviewed journals.

Building on the Work of Others

The great scientist Isaac Newton once said: "To explain all nature is too difficult a task for any one man or even for any one age. 'Tis much better to do a little with certainty, and leave the rest for others that come after you. . . ." Anyone who becomes a scientist, driven by curiosity about how nature works, is sure to benefit greatly from the rich storehouse of discoveries by others who have come before. In fact, Hopi Hoekstra's experiment benefited from the work of another

researcher, D. W. Kaufman, 40 years earlier. You can study the design of Kaufman's experiment and interpret the results in the **Scientific Skills Exercise**.

Scientific results are continually scrutinized through the repetition of observations and experiments. Scientists working in the same research field often check one another's claims by attempting to confirm observations or repeat experiments. If scientific colleagues cannot repeat experimental findings, this failure may reflect some underlying weakness in the original claim, which will then have to be revised. In this sense, science polices itself. Integrity and adherence to high professional standards in reporting results are central to the scientific endeavor, since the validity of experimental data is key to designing further lines of inquiry.

It is not unusual for several scientists to converge on the same research question. Some scientists enjoy the challenge of being first with an important discovery or key experiment, while others derive more satisfaction from cooperating with fellow scientists working on the same problem.

Cooperation is facilitated when scientists use the same organism. Often it is a widely used **model organism**—a species that is easy to grow in the lab and lends itself particularly well to the questions being investigated. Because all species are evolutionarily related, such an organism may be viewed as a model for understanding the biology of other species and their diseases. For example, genetic studies of the fruit fly *Drosophila melanogaster* have taught us a lot about how genes work in other species, even humans. Some other popular model organisms are the mustard plant *Arabidopsis thaliana*, the soil worm *Caenorhabditis elegans*, the zebrafish *Danio rerio*, the mouse *Mus musculus*, and the bacterium *Escherichia coli*. As you read through this book, note the many contributions that these and other model organisms have made to the study of life.

Biologists may approach interesting questions from different angles. Some biologists focus on ecosystems, while others study natural phenomena at the level of organisms or cells. This text is divided into units that look at biology at different levels and investigate problems through different approaches. Yet any given problem can be addressed from many perspectives, which in fact complement each other. For example, Hoekstra's work uncovered at least one genetic mutation that underlies the differences between beach and inland mouse coloration. Her lab includes biologists specializing in different biological levels, allowing links to be made between the evolutionary adaptations she focuses on and their molecular basis in DNA sequences.

As a biology student, you can benefit from making connections between the different levels of biology. You can develop this skill by noticing when certain topics crop up again and again in different units. One such topic is sickle-cell disease, a well-understood genetic condition that is prevalent among native inhabitants of Africa and other warm regions and their

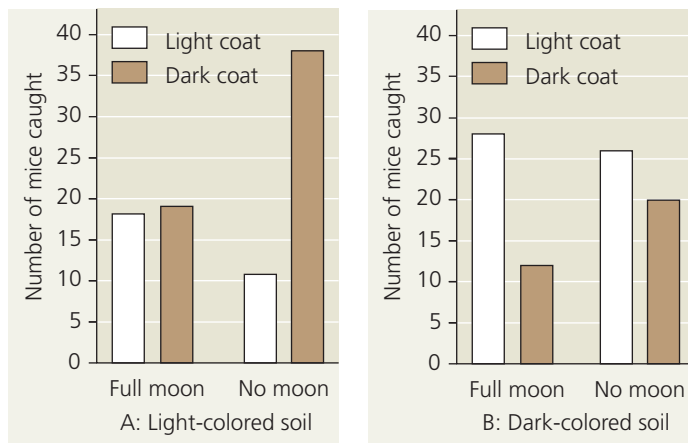
SCIENTIFIC SKILLS EXERCISE

Interpreting a Pair of Bar Graphs

How Much Does Camouflage Affect Predation on Mice by Owls With and Without Moonlight? D. W. Kaufman hypothesized that the extent to which the coat color of a mouse contrasted with the color of its surroundings would affect the rate of nighttime predation by owls. He also hypothesized that the contrast would be affected by the amount of moonlight. In this exercise, you will analyze data from his studies of owl predation on mice that tested these hypotheses.

How the Experiment Was Done Pairs of mice (*Peromyscus polionotus*) with different coat colors, one light brown and one dark brown, were released simultaneously into an enclosure that contained a hungry owl. The researcher recorded the color of the mouse that was first caught by the owl. If the owl did not catch either mouse within 15 minutes, the test was recorded as a zero. The release trials were repeated multiple times in enclosures with either a dark-colored soil surface or a light-colored soil surface. The presence or absence of moonlight during each assay was recorded.

Data from the Experiment



Data from D. W. Kaufman, Adaptive coloration in *Peromyscus polionotus*: Experimental selection by owls, *Journal of Mammalogy* 55:271–283 (1974).

INTERPRET THE DATA

1. First, make sure you understand how the graphs are set up. Graph A shows data from the light-colored soil enclosure and graph B from the dark-colored enclosure, but in all other respects the graphs are the same. (a) There is more than one independent variable in these graphs. What are the independent variables, the variables that were tested by the researcher? Which axis of the graphs has the independent variables? (b) What is the dependent variable, the response to the variables being tested? Which axis of the graphs has the dependent variable?
2. (a) How many dark brown mice were caught in the light-colored soil enclosure on a moonlit night? (b) How many dark brown mice were caught in the dark-colored soil enclosure on a moonlit night? (c) On a moonlit night, would a dark brown mouse be more likely to escape predation by owls on dark- or light-colored soil? Explain your answer.
3. (a) Is a dark brown mouse on dark-colored soil more likely to escape predation under a full moon or with no moon? (b) What about a light brown mouse on light-colored soil? Explain.
4. (a) Under which conditions would a dark brown mouse be most likely to escape predation at night? (b) A light brown mouse?
5. (a) What combination of independent variables led to the highest predation level in enclosures with light-colored soil? (b) What combination of independent variables led to the highest predation level in enclosures with dark-colored soil?
6. Thinking about your answers to question 5, provide a simple statement describing conditions that are especially deadly for either color of mouse.
7. Combining the data from both graphs, estimate the number of mice caught in moonlight versus no-moonlight conditions. Which condition is optimal for predation by the owl? Explain.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.



descendants. Sickle-cell disease will appear in several units of the text, each time addressed at a new level. In addition, we have designed a number of figures that make connections between the content in different chapters, as well as questions that ask you to make the connections yourselves. We hope these features will help you integrate the material you're learning and enhance your enjoyment of biology by encouraging you to keep the big picture in mind.

Science, Technology, and Society

The research community is part of society at large, and the relationship of science to society becomes clearer when we add technology to the picture (see Figure 1.23). Though

science and technology sometimes employ similar inquiry patterns, their basic goals differ. The goal of science is to understand natural phenomena, while that of **technology** is to *apply* scientific knowledge for some specific purpose. Biologists and other scientists usually speak of “discoveries,” while engineers and other technologists more often speak of “inventions.” Because scientists put new technology to work in their research, science and technology are interdependent.

The potent combination of science and technology can have dramatic effects on society. Sometimes, the applications of basic research that turn out to be the most beneficial come out of the blue, from completely unanticipated observations in the course of scientific exploration. For example, discovery of the structure of DNA by Watson and

▼ Figure 1.26 DNA technology and forensics. In 2011, forensic analysis of DNA samples from a crime scene led to the release of Michael Morton from prison after he had served nearly 25 years for a crime he didn't commit, the brutal murder of his wife. The DNA analysis linked another man, also charged in a second murder, to the crime. The photo shows Mr. Morton hugging his parents after his conviction was overturned. The details of forensic analysis of DNA will be described in Chapter 19.



Crick 60 years ago and subsequent achievements in DNA science led to the technologies of DNA manipulation that are transforming applied fields such as medicine, agriculture, and forensics (**Figure 1.26**). Perhaps Watson and Crick envisioned that their discovery would someday lead to important applications, but it is unlikely that they could have predicted exactly what all those applications would be.

The directions that technology takes depend less on the curiosity that drives basic science than on the current needs and wants of people and on the social environment of the times. Debates about technology center more on “*should* we do it” than “*can* we do it.” With advances in technology come difficult choices. For example, under what circumstances is it acceptable to use DNA technology to find out if particular people have genes for hereditary diseases? Should such tests always be voluntary, or are there circumstances when genetic testing should be mandatory? Should insurance companies or employers have access to the information, as they do for many other types of personal health data? These questions are becoming much more urgent as the sequencing of individual genomes becomes quicker and cheaper.

Ethical issues raised by such questions have as much to do with politics, economics, and cultural values as with science and technology. All citizens—not only professional scientists—have a responsibility to be informed about how science works and about the potential benefits and risks of technology. The relationship between science, technology, and society increases the significance and value of any biology course.

The Value of Diverse Viewpoints in Science

Many of the technological innovations with the most profound impact on human society originated in settlements along trade routes, where a rich mix of different cultures ignited new ideas. For example, the printing press, which helped spread knowledge to all social classes, was invented by the German Johannes Gutenberg around 1440. This invention relied on several innovations from China, including paper and ink. Paper traveled along trade routes from China to Baghdad, where technology was developed for its mass production. This technology then migrated to Europe, as did water-based ink from China, which was modified by Gutenberg to become oil-based ink. We have the cross-fertilization of diverse cultures to thank for the printing press, and the same can be said for other important inventions.

Along similar lines, science stands to gain much from embracing a diversity of backgrounds and viewpoints among its practitioners. But just how diverse a population are scientists in relation to gender, race, ethnicity, and other attributes?

The scientific community reflects the cultural standards and behaviors of the society around it. It is therefore not surprising that until recently, women and certain minorities have faced huge obstacles in their pursuit to become professional scientists in many countries around the world. Over the past 50 years, changing attitudes about career choices have increased the proportion of women in biology and some other sciences, so that now women constitute roughly half of undergraduate biology majors and biology Ph.D. students.

The pace has been slow at higher levels in the profession, however, and women and many racial and ethnic groups are still significantly underrepresented in many branches of science and technology. This lack of diversity hampers the progress of science. The more voices that are heard at the table, the more robust, valuable, and productive the scientific interchange will be. The authors of this text welcome all students to the community of biologists, wishing you the joys and satisfactions of this exciting field of science.

CONCEPT CHECK 1.4

1. How does science differ from technology?
2. **MAKE CONNECTIONS** ► The gene that causes sickle-cell disease is present in a higher percentage of residents of sub-Saharan Africa than among those of African descent living in the United States. Even though this gene causes sickle-cell disease, it also provides some protection from malaria, a serious disease that is widespread in sub-Saharan Africa but absent in the United States. Discuss an evolutionary process that could account for the different percentages of the sickle-cell gene among residents of the two regions. (See Concept 1.2.)

For suggested answers, see Appendix A.

1 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 1.1

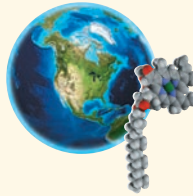
The study of life reveals unifying themes (pp. 52–59)

Organization Theme: New Properties Emerge at Successive Levels of Biological Organization

- The hierarchy of life unfolds as follows:
biosphere > ecosystem > community > population > organism > organ system > organ > tissue > cell > organelle > molecule > atom.
With each step upward from atoms, new **emergent properties** result from interactions among components at the lower levels. In an approach called **reductionism**, complex systems are broken down to simpler components that are more manageable to study. In **systems biology**, scientists attempt to model the dynamic behavior of whole biological systems by studying the interactions among the system's parts.
- Structure and function are correlated at all levels of biological organization. The cell, an organism's basic unit of structure and function, is the lowest level that can perform all activities required for life. Cells are either prokaryotic or eukaryotic. **Eukaryotic cells** contain membrane-enclosed organelles, including a DNA-containing nucleus. **Prokaryotic cells** lack such organelles.

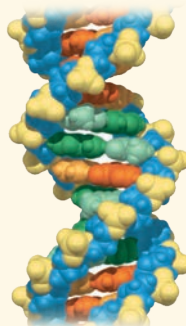


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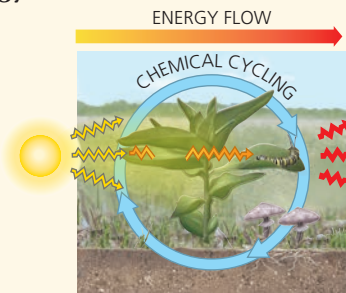
Information Theme: Life's Processes Involve the Expression and Transmission of Genetic Information

- Genetic information is encoded in the nucleotide sequences of **DNA**. It is DNA that transmits heritable information from parents to offspring. DNA sequences (called **genes**) program a cell's protein production by being transcribed into mRNA and then translated into specific proteins, a process called **gene expression**. Gene expression also produces RNAs that are not translated into protein but serve other important functions. **Genomics** is the large-scale analysis of the DNA sequences of a species (its **genome**) as well as the comparison of genomes between species. **Bioinformatics** uses computational tools to deal with huge volumes of sequence data.



Energy and Matter Theme: Life Requires the Transfer and Transformation of Energy and Matter

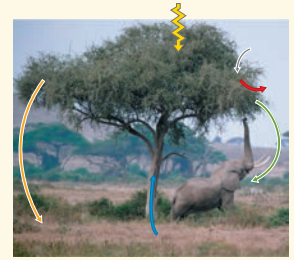
- Energy flows through an ecosystem. All organisms must perform work, which requires energy. **Producers** convert energy from sunlight to chemical energy, some of which is then passed on to **consumers**. (The rest is lost from the ecosystem as heat.) Chemicals cycle between organisms and the environment.



Interactions Theme: From Molecules to Ecosystems, Interactions Are Important in Biological Systems

- In **feedback regulation**, a process is regulated by its output or end product. In negative feedback, accumulation of the end product slows its production. In positive feedback, an end product speeds up its own production.

- Organisms interact continuously with physical factors. Plants take up nutrients from the soil and chemicals from the air and use energy from the sun.

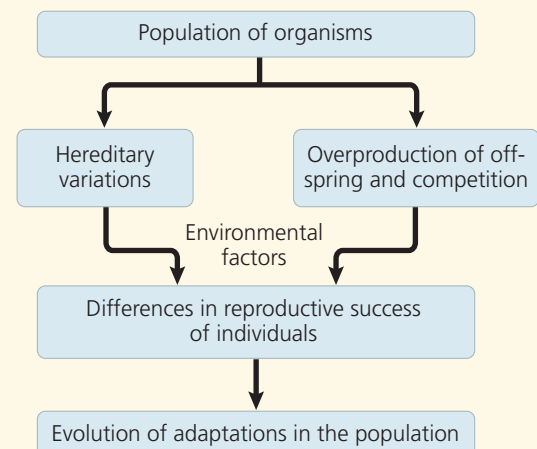
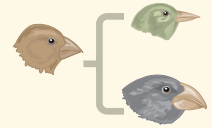


- ? Thinking about the muscles and nerves in your hand, how does the activity of text messaging reflect the four themes of biology described in this section?

CONCEPT 1.2

The Core Theme: Evolution accounts for the unity and diversity of life (pp. 59–64)

- Evolution**, the process of change that has transformed life on Earth, accounts for the unity and diversity of life. It also explains evolutionary adaptation—the match of organisms to their environments.
- Biologists classify species according to a system of broader and broader groups. Domain **Bacteria** and domain **Archaea** consist of prokaryotes. Domain **Eukarya**, the eukaryotes, includes various groups of protists and the kingdoms Plantae, Fungi, and Animalia. As diverse as life is, there is also evidence of remarkable unity, revealed in the similarities between different species.
- Darwin proposed **natural selection** as the mechanism for evolutionary adaptation of populations to their environments. Natural selection is the evolutionary process that occurs when a population is exposed to environmental factors that consistently cause individuals with certain heritable traits to have greater reproductive success than do individuals with other heritable traits.



- Each species is one twig of a branching tree of life extending back in time through more and more remote ancestral species. All of life is connected through its long evolutionary history.

- ? How could natural selection have led to the evolution of adaptations such as camouflaging coat color in beach mice?

CONCEPT 1.3

In studying nature, scientists make observations and form and test hypotheses (pp. 64–70)

- In scientific **inquiry**, scientists make and record observations (collect **data**) and use **inductive reasoning** to draw a general conclusion, which can be developed into a testable **hypothesis**. **Deductive reasoning** makes predictions that can be used to

test hypotheses. Hypotheses must be testable; science can address neither the possibility of supernatural phenomena nor the validity of religious beliefs. Hypotheses can be tested by conducting **experiments** or, when that is not possible, by making observations. In the process of science, the core activity is testing ideas. This endeavor is influenced by exploration and discovery, community analysis and feedback, and societal outcomes.

- **Controlled experiments**, such as the investigation of coat color in mouse populations, are designed to demonstrate the effect of one **variable** by testing control groups and experimental groups that differ in only that one variable.
- A scientific **theory** is broad in scope, generates new hypotheses, and is supported by a large body of evidence.

? What are the roles of gathering and interpreting data in the process of scientific inquiry?

CONCEPT 1.4

Science benefits from a cooperative approach and diverse viewpoints (pp. 70–72)

- Science is a social activity. The work of each scientist builds on the work of others who have come before. Scientists must be able to repeat each other's results, and integrity is key. Biologists approach questions at different levels; their approaches complement each other.
- **Technology** consists of any method or device that applies scientific knowledge for some specific purpose that affects society. The impact of basic research is not always immediately obvious.
- Diversity among scientists promotes progress in science.

? Explain why different approaches and diverse backgrounds among scientists are important.

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. **DRAW IT** With rough sketches, draw a biological hierarchy similar to the one in Figure 1.3 but using a coral reef as the ecosystem, a fish as the organism, its stomach as the organ, and DNA as the molecule. Include all levels in the hierarchy.
9. **EVOLUTION CONNECTION** A typical prokaryotic cell has about 3,000 genes in its DNA, while a



PRACTICE TEST
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human cell has almost 21,000 genes. About 1,000 of these genes are present in both types of cells. Based on your understanding of evolution, explain how such different organisms could have this same subset of 1,000 genes. What sorts of functions might these shared genes have?

10. **SCIENTIFIC INQUIRY** Based on the results of the mouse coloration case study, suggest another hypothesis researchers might use to further study the role of predators in the natural selection process.
11. **SCIENTIFIC INQUIRY** Scientists search the scientific literature by means of electronic databases such as PubMed, a free online database maintained by the National Center for Biotechnology Information. Use PubMed to find the abstract of a scientific article that Hopi Hoekstra published in 2015 or later.
12. **WRITE ABOUT A THEME: EVOLUTION** In a short essay (100–150 words), discuss Darwin's view of how natural selection resulted in both unity and diversity of life on Earth. Include in your discussion some of his evidence. (See a suggested grading rubric and tips for writing good essays in the Study Area of MasteringBiology under "Write About a Theme.")
13. **SYNTHESIZE YOUR KNOWLEDGE**



Can you pick out the mossy leaf-tailed gecko lying against the tree trunk in this photo? How is the appearance of the gecko a benefit in terms of survival? Given what you learned about evolution, natural selection, and genetic information in this chapter, describe how the gecko's coloration might have evolved.

For selected answers, see Appendix A.

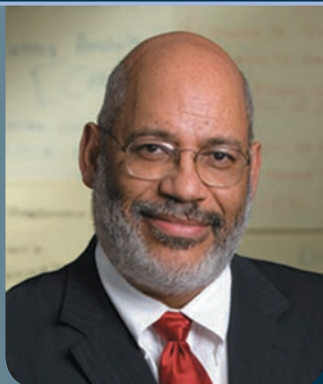


For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

UNIT 1

THE ROLE OF CHEMISTRY IN BIOLOGY

Born in Baton Rouge, Louisiana, Lovell Jones was one of the first students to integrate the schools in that city. He went on to major in biology at California State University at Hayward (now CSU East Bay). He then earned a Ph.D. in Zoology with an emphasis in endocrinology and tumor biology from the University of California, Berkeley, in 1977. As a postdoctoral fellow at the University of California, San Francisco, he carried out research on the effects of estrogen on breast cancer. In 1980, after his post-doc, he moved to the MD Anderson Cancer Center as an assistant professor in the Department of Gynecology and Biochemistry, where he remained until his retirement in 2013. In addition to making major contributions to our understanding of the role of steroid hormones in reproductive cancers, Dr. Jones is considered a pioneer in addressing health inequities and social justice. He has continued the latter work during his retirement. During his long, distinguished career, Dr. Jones has received numerous awards and has been commended in the U.S. House of Representatives for his lifelong work.



An Interview with Lovell Jones

What did you study in graduate school?

I got my Ph.D. at UC Berkeley, working with Howard Bern on the effects of hormones on cervical and vaginal development. I developed an interest in the effects of an artificial estrogen called diethylstilbestrol (DES), a medication used to stop spontaneous abortion in pregnant women. Women that used DES during their first trimester gave birth to girls who had an increased incidence of a rare form of cervical cancer at a very early age, as young as 7. At that time, it was thought that DES was acting as a chemical to initiate cancer, but I thought it was a hormonal action because it was mimicking estrogen, and I wanted to test that using mice. By expos-

ing mice to natural estrogen when their cervical and vaginal tracts were developing, I showed that this exposure produced related types of tumors.

How did you continue to work on the relationship between hormones and cancer?

After I got my Ph.D., I worked with Finn Siiteri at UCSF, who was an expert on endocrine-related hormones but had no cancer knowledge. He brought me on because I had shown that hormones could initiate cancer. I started to work on finding a way to measure estrogen levels as a risk factor for developing breast cancer. I found that the level of free estrogen—estrogen that isn't bound to other molecules—was the risk factor because only free estrogen was able to function. At this point, I was recruited to MD Anderson Cancer Center to develop hormonal therapies using agents that blocked estrogens. We developed treatments for lung cancer, head and neck cancers, and salivary cancers. At the same time, I was looking at long-term effects of estrogens on neonatal development and developing a method to use hormone levels to predict breast cancer risk.

Why study biochemistry, and what are the most exciting avenues of biochemical research?

Studying biochemistry in a holistic way is going to be the key to the answers we need. We have to get out of our silos and do what some people call trans-disciplinary research. As far as future research goes, the most exciting area is precision medicine, an approach to treatment that considers a person's genes, environment, and lifestyle. We can now get away from looking at individuals in terms of their race, which is not a biological construct, and instead look at them as members of populations: subgroups that have a particular history together. For example, one of the highest incidences of pre-menopausal breast cancer is among blacks and whites in the Chesapeake Bay area. Why? Well, most white females who came into Maryland during colonial times were Scotch-Irish Catholics who arrived as indentured servants, a higher form of slaves. Roughly a third of them had interactions with African slaves. So a lot of people in that area have genes that came from Africa, and some people who look white have black ancestry. A disease called triple-negative breast cancer, where tumor cells are missing three types of receptors, is spoken of as a "black cancer." But it's really just that a group of genes that arose in one place in Africa leads to early onset of breast cancer. Also, triple-negative breast cancer is a disease of the young, not of black females in particular. So that's why I say precision medicine will need to analyze all of an individual's genes and consider their evolutionary history rather than just assigning them to one race or another.

What is your advice to an undergraduate considering a career in biology?

Find a good mentor. Some of the greatest mentors put their students ahead of themselves. Howard Bern, my Ph.D. advisor, said that the greatest contribution to science is not your papers or grants, but the people you leave behind.

"Studying biochemistry in a holistic way is going to be the key to the answers we need."

▼ **Dr. Lovell Jones in his lab, where he has studied the relationship between hormones and cancers.**



Atoms and Molecules

2



▲ **Figure 2.1** What weapon do these wood ants use to deter predators?

KEY CONCEPTS

- 2.1** Matter consists of chemical elements in pure form and in combinations called compounds
- 2.2** An element's properties depend on the structure of its atoms
- 2.3** The formation and function of molecules depend on chemical bonding between atoms
- 2.4** Chemical reactions make and break chemical bonds



A Chemical Connection to Biology

Like other animals, ants have structures and mechanisms that defend them from attack. Wood ants live in colonies of hundreds or thousands, and the colony as a whole has a particularly effective way of dealing with enemies. When threatened from above, the ants shoot volleys of formic acid into the air from their abdomens, and the acid bombards the potential predator, such as a hungry bird. Formic acid is produced by many species of ants and in fact got its name from the Latin word for ant, *formica*. For quite a few ant species, the formic acid isn't shot out, but probably serves as a disinfectant that protects the ants against microbial parasites. Scientists have long known that chemicals play a major role in insect communication, the attraction of mates, and defense against predators.

Research on ants and other insects is a good example of how relevant chemistry is to the study of life. Unlike college courses, nature is not neatly packaged into individual sciences—biology, chemistry, physics, and so forth. Biologists specialize in the study of life, but organisms and their environments are natural systems to which the concepts of chemistry and physics apply. Biology is multidisciplinary.

This unit of chapters introduces some basic concepts of chemistry that apply to the study of life. Somewhere in the transition from molecules to cells, we will cross the blurry boundary between nonlife and life. This chapter focuses on the chemical components that make up all matter.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.

 **Get Ready for This Chapter**



Interview with Deborah Gordon: Investigating ant behavior

CONCEPT 2.1

Matter consists of chemical elements in pure form and in combinations called compounds

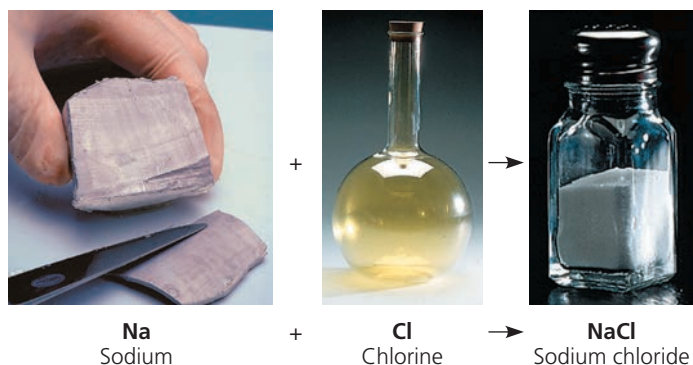
Organisms are composed of **matter**, which is anything that takes up space and has mass.* Matter exists in many forms. Rocks, metals, oils, gases, and living organisms are a few examples of what seems to be an endless assortment of matter.

Elements and Compounds

Matter is made up of elements. An **element** is a substance that cannot be broken down to other substances by chemical reactions. Today, chemists recognize 92 elements occurring in nature; gold, copper, carbon, and oxygen are examples. Each element has a symbol, usually the first letter or two of its name. Some symbols are derived from Latin or German; for instance, the symbol for sodium is Na, from the Latin word *natrum*.

A **compound** is a substance consisting of two or more different elements combined in a fixed ratio. Table salt, for example, is sodium chloride (NaCl), a compound composed of the elements sodium (Na) and chlorine (Cl) in a 1:1 ratio. Pure sodium is a metal, and pure chlorine is a poisonous gas. When chemically combined, however, sodium and chlorine form an edible compound. Water (H₂O), another compound, consists of the elements hydrogen (H) and oxygen (O) in a 2:1 ratio. These are simple examples of organized matter having emergent properties: A compound has characteristics different from those of its elements (**Figure 2.2**).

▼ **Figure 2.2 The emergent properties of a compound.** The metal sodium combines with the poisonous gas chlorine, forming the edible compound sodium chloride, or table salt.



*In everyday language we tend to substitute the term weight for mass, although the two are not identical. Mass is the amount of matter in an object, whereas the weight of an object is how strongly that mass is pulled by gravity. The weight of an astronaut walking on the moon is approximately $\frac{1}{6}$ the astronaut's weight on Earth, but his or her mass is the same. However, as long as we are earthbound, the weight of an object is a measure of its mass; in everyday language, therefore, we tend to use the terms interchangeably.

The Elements of Life

Of the 92 natural elements, about 20–25% are **essential elements** that an organism needs to live a healthy life and reproduce. The essential elements are similar among organisms, but there is some variation—for example, humans need 25 elements, but plants need only 17.

Just four elements—oxygen (O), carbon (C), hydrogen (H), and nitrogen (N)—make up approximately 96% of living matter. Calcium (Ca), phosphorus (P), potassium (K), sulfur (S), and a few other elements account for most of the remaining 4% or so of an organism's mass. **Trace elements** are required by an organism in only minute quantities. Some trace elements, such as iron (Fe), are needed by all forms of life; others are required only by certain species. For example, in vertebrates (animals with backbones), the element iodine (I) is an essential ingredient of a hormone produced by the thyroid gland. A daily intake of only 0.15 milligram (mg) of iodine is adequate for normal activity of the human thyroid. An iodine deficiency in the diet causes the thyroid gland to grow to abnormal size, a condition called goiter. Consuming seafood or iodized salt reduces the incidence of goiter. Relative amounts of all the elements in the human body are listed in **Table 2.1**.

Some naturally occurring elements are toxic to organisms. In humans, for instance, the element arsenic has been linked to numerous diseases and can be lethal. In some areas of the world, arsenic occurs naturally and can make its way into the groundwater. As a result of using water from drilled wells in southern Asia, millions of people have been inadvertently exposed to arsenic-laden water. Efforts are under way to reduce arsenic levels in their water supply.

Table 2.1 Elements in the Human Body

Element	Symbol	Percentage of Body Mass (including water)	
Oxygen	O	65.0%	96.3%
Carbon	C	18.5%	
Hydrogen	H	9.5%	
Nitrogen	N	3.3%	
Calcium	Ca	1.5%	3.7%
Phosphorus	P	1.0%	
Potassium	K	0.4%	
Sulfur	S	0.3%	
Sodium	Na	0.2%	
Chlorine	Cl	0.2%	
Magnesium	Mg	0.1%	
Trace elements (less than 0.01% of mass): Boron (B), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), silicon (Si), tin (Sn), vanadium (V), zinc (Zn)			

INTERPRET THE DATA ► Given the makeup of the human body, what compound do you think accounts for the high percentage of oxygen?

▼ **Figure 2.3 Serpentine plant community.** These plants are growing on serpentine soil, which contains elements that are usually toxic to plants. The insets show a close-up of serpentine rock and one of the plants, a Tiburon Mariposa lily (*Calochortus tiburonensis*). This specially-adapted species is found only on this one hill in Tiburon, a peninsula that juts into San Francisco Bay.



Case Study: Evolution of Tolerance to Toxic Elements

EVOLUTION Some species have become adapted to environments containing elements that are usually toxic; an example is serpentine plant communities. Serpentine is a jade-like mineral that contains elevated concentrations of elements such as chromium, nickel, and cobalt. Although most plants cannot survive in soil that forms from serpentine rock, a small number of plant species have adaptations that allow them to do so (**Figure 2.3**). Presumably, variants of ancestral, nonserpentine species arose that could survive in serpentine soils, and subsequent natural selection resulted in the distinctive array of species we see in these areas today. Researchers are studying whether serpentine-adapted plants could be used to take up toxic heavy metals in contaminated areas, concentrating them for safer disposal.

CONCEPT CHECK 2.1

1. **MAKE CONNECTIONS** ► Explain how table salt has emergent properties. (See Concept 1.1.)
2. Is a trace element an essential element? Explain.
3. **WHAT IF?** ► In humans, potassium helps to regulate the acid-base balance in blood and tissues. It also plays a key role in the transmission of neural signals, resulting in the contraction of skeletal and smooth muscles. What might be the effects of a potassium deficiency?
4. **MAKE CONNECTIONS** ► Explain how natural selection might have played a role in the evolution of species that are tolerant of serpentine soils. (Review Concept 1.2.)

For suggested answers, see Appendix A.

CONCEPT 2.2

An element's properties depend on the structure of its atoms

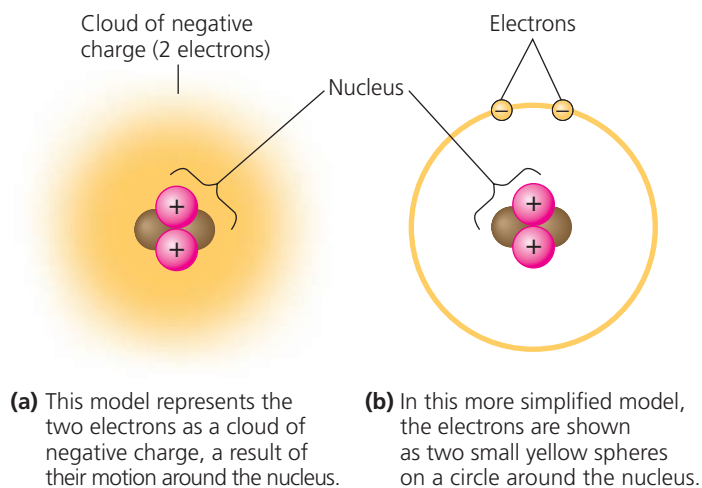
Each element consists of a certain type of atom that is different from the atoms of any other element. An **atom** is the smallest unit of matter that still retains the properties of an element. Atoms are so small that it would take about a million of them to stretch across the period printed at the end of this sentence. We symbolize atoms with the same abbreviation used for the element that is made up of those atoms. For example, the symbol C stands for both the element carbon and a single carbon atom.

Subatomic Particles

Although the atom is the smallest unit having the properties of an element, these tiny bits of matter are composed of even smaller parts, called *subatomic particles*. Using high-energy collisions, physicists have produced more than 100 types of particles from the atom, but only three kinds of particles are relevant here: **neutrons**, **protons**, and **electrons**. Protons and electrons are electrically charged. Each proton has one unit of positive charge, and each electron has one unit of negative charge. A neutron, as its name implies, is electrically neutral.

Protons and neutrons are packed together tightly in a dense core, or **atomic nucleus**, at the center of an atom; protons give the nucleus a positive charge. The rapidly moving electrons form a “cloud” of negative charge around the nucleus, and it is the attraction between opposite charges that keeps the electrons in the vicinity of the nucleus. **Figure 2.4** shows

▼ **Figure 2.4 Simplified models of a helium (He) atom.** The helium nucleus consists of 2 neutrons (brown) and 2 protons (pink). Two electrons (yellow) exist outside the nucleus. These models are not to scale; they greatly overestimate the size of the nucleus in relation to the electron cloud.



two commonly used models of the structure of the helium atom as an example.

The neutron and proton are almost identical in mass, each about 1.7×10^{-24} gram (g). Grams and other conventional units are not very useful for describing the mass of objects that are so minuscule. Thus, for atoms and subatomic particles (and for molecules, too), we use a unit of measurement called the **dalton**, in honor of John Dalton, the British scientist who helped develop atomic theory around 1800. (The dalton is the same as the *atomic mass unit*, or *amu*, a unit you may have encountered elsewhere.) Neutrons and protons have masses close to 1 dalton. Because the mass of an electron is only about 1/2,000 that of a neutron or proton, we can ignore electrons when computing the total mass of an atom.

Atomic Number and Atomic Mass

Atoms of the various elements differ in their number of subatomic particles. All atoms of a particular element have the same number of protons in their nuclei. This number of protons, which is unique to that element, is called the **atomic number** and is written as a subscript to the left of the symbol for the element. The abbreviation ${}_2\text{He}$, for example, tells us that an atom of the element helium has 2 protons in its nucleus. Unless otherwise indicated, an atom is neutral in electrical charge, which means that its protons must be balanced by an equal number of electrons. Therefore, the atomic number tells us the number of protons and also the number of electrons in an electrically neutral atom.

We can deduce the number of neutrons from a second quantity, the **mass number**, which is the total number of protons and neutrons in the nucleus of an atom. The mass number is written as a superscript to the left of an element's symbol. For example, we can use this shorthand to write an atom of helium as ${}_2^4\text{He}$. Because the atomic number indicates how many protons there are, we can determine the number of neutrons by subtracting the atomic number from the mass number. In our example, the helium atom ${}_2^4\text{He}$ has 2 neutrons. For sodium (Na):

$$\begin{array}{l} \text{Mass number} = \text{number of protons} + \text{neutrons} \\ \quad \quad \quad = 23 \text{ for sodium} \\ \swarrow \quad \quad \quad \searrow \\ {}_{11}^{23}\text{Na} \\ \swarrow \quad \quad \quad \searrow \\ \text{Atomic number} = \text{number of protons} \\ \quad \quad \quad = \text{number of electrons in a neutral atom} \\ \quad \quad \quad = 11 \text{ for sodium} \end{array}$$

$$\begin{array}{l} \text{Number of neutrons} = \text{mass number} - \text{atomic number} \\ \quad \quad \quad = 23 - 11 = 12 \text{ for sodium} \end{array}$$

The simplest atom is hydrogen ${}_1^1\text{H}$, which has no neutrons; it consists of a single proton with a single electron.

Because the contribution of electrons to mass is negligible, almost all of an atom's mass is concentrated in its nucleus.

Neutrons and protons each have a mass very close to 1 dalton, so the mass number is close to, but slightly different from, the total mass of an atom, called its **atomic mass**. For example, the mass number of sodium (${}_{11}^{23}\text{Na}$) is 23, but its atomic mass is 22.9898 daltons.



Animation: Atomic Number and Atomic Mass

Isotopes

All atoms of a given element have the same number of protons, but some atoms have more neutrons than other atoms of the same element and therefore have greater mass. These different atomic forms of the same element are called **isotopes** of the element. In nature, an element may occur as a mixture of its isotopes. As an example, the element carbon, which has the atomic number 6, has three naturally occurring isotopes. The most common isotope is carbon-12, ${}^{12}_6\text{C}$, which accounts for about 99% of the carbon in nature. The isotope ${}^{12}_6\text{C}$ has 6 neutrons. Most of the remaining 1% of carbon consists of atoms of the isotope ${}^{13}_6\text{C}$, with 7 neutrons. A third, even rarer isotope, ${}^{14}_6\text{C}$, has 8 neutrons. Notice that all three isotopes of carbon have 6 protons; otherwise, they would not be carbon. Although the isotopes of an element have slightly different masses, they behave identically in chemical reactions. (For an element with more than one naturally occurring isotope, the atomic mass is an average of those isotopes, weighted by their abundance. Thus carbon has an atomic mass of 12.01 daltons.)

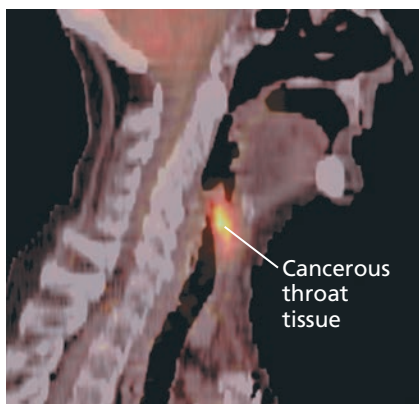
Both ${}^{12}_6\text{C}$ and ${}^{13}_6\text{C}$ are stable isotopes, meaning that their nuclei do not have a tendency to lose subatomic particles, a process called decay. The isotope ${}^{14}_6\text{C}$, however, is unstable, or radioactive. A **radioactive isotope** is one in which the nucleus decays spontaneously, giving off particles and energy. When the radioactive decay leads to a change in the number of protons, it transforms the atom to an atom of a different element. For example, when an atom of carbon-14 (${}^{14}_6\text{C}$) decays, it loses a proton, becoming an atom of nitrogen (${}^{14}_7\text{N}$). Radioactive isotopes have many useful applications in biology.

Radioactive Tracers

Radioactive isotopes are often used as diagnostic tools in medicine. Cells can use radioactive atoms just as they would use nonradioactive isotopes of the same element. The radioactive isotopes are incorporated into biologically active molecules, which are then used as tracers to track atoms during metabolism, the chemical processes of an organism. For example, certain kidney disorders are diagnosed by injecting small doses of radioactively labeled substances into the blood and then analyzing the tracer molecules excreted in the urine. Radioactive tracers are also used in combination with sophisticated imaging instruments, such as PET scanners

► **Figure 2.5** A PET scan, a medical use for radioactive isotopes.

PET, an acronym for positron-emission tomography, detects locations of intense chemical activity in the body. The bright yellow spot marks an area with an elevated level of radioactively labeled glucose, which in turn indicates high metabolic activity, a hallmark of cancerous tissue.



that can monitor growth and metabolism of cancers in the body (**Figure 2.5**).

Although radioactive isotopes are very useful in biological research and medicine, radiation from decaying isotopes also poses a hazard to life by damaging cellular molecules. The severity of this damage depends on the type and amount of radiation an organism absorbs. One of the most serious environmental threats is radioactive fallout from nuclear accidents. The doses of most isotopes used in medical diagnosis, however, are relatively safe.

Radiometric Dating

EVOLUTION Researchers measure radioactive decay in fossils to date these relics of past life. Fossils provide a large body of evidence for evolution, documenting differences between organisms from the past and those living at present and giving us insight into species that have disappeared over time. While the layering of fossil beds establishes that deeper fossils are older than more shallow ones, the actual age (in years) of the fossils in each layer cannot be determined by position alone. This is where radioactive isotopes come in.

A “parent” isotope decays into its “daughter” isotope at a fixed rate, expressed as the **half-life** of the isotope—the time it takes for 50% of the parent isotope to decay. Each radioactive isotope has a characteristic half-life that is not affected by temperature, pressure, or any other environmental variable. Using a process called **radiometric dating**, scientists measure the ratio of different isotopes and calculate how many half-lives (in years) have passed since an organism was fossilized or a rock was formed. Half-life values range from very short for some isotopes, measured in seconds or days, to extremely long—uranium-238 has a half-life of 4.5 billion years! Each isotope can best “measure” a particular range of years: Uranium-238 was used to determine that moon rocks are approximately 4.5 billion years old, similar to the estimated age of Earth. In the **Scientific Skills Exercise**, you can work with data from an experiment that used carbon-14 to determine the age of an important fossil. (Figure 25.6 explains more about radiometric dating of fossils.)

The Energy Levels of Electrons

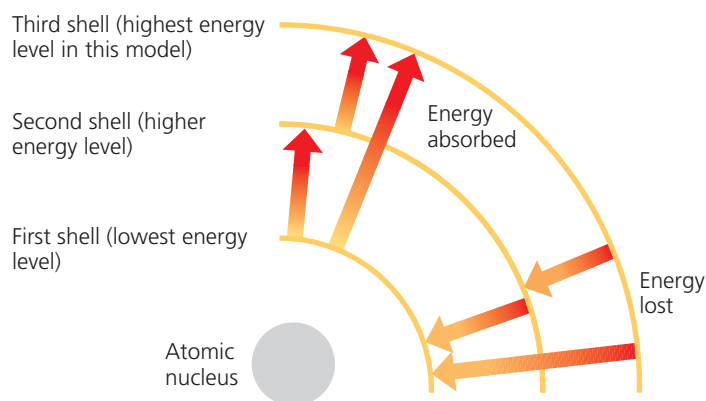
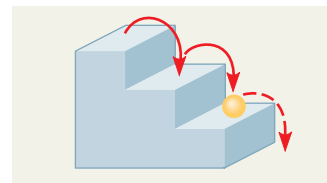
The simplified models of the atom in Figure 2.4 greatly exaggerate the size of the nucleus relative to that of the whole atom. If an atom of helium were the size of a typical football stadium, the nucleus would be the size of a pencil eraser in the center of the field. Moreover, the electrons would be like two tiny gnats buzzing around the stadium. Atoms are mostly empty space. When two atoms approach each other during a chemical reaction, their nuclei do not come close enough to interact. Of the three subatomic particles we have discussed, only electrons are directly involved in chemical reactions.

An atom’s electrons vary in the amount of energy they possess. **Energy** is defined as the capacity to cause change—for instance, by doing work. **Potential energy** is the energy that matter possesses because of its location or structure. For example, water in a reservoir on a hill has potential energy because of its altitude. When the gates of the reservoir’s dam are opened and the water runs downhill, the energy can be used to do work, such as moving the blades of turbines to generate electricity. Because energy has been expended, the water has less energy at the bottom of the hill than it did in the reservoir. Matter has a natural tendency to move toward the lowest possible state of potential energy; in our example, the water runs downhill. To restore the potential energy of a reservoir, work must be done to elevate the water against gravity.

The electrons of an atom have potential energy due to their distance from the nucleus (**Figure 2.6**). The negatively charged

▼ **Figure 2.6** Energy levels of an atom’s electrons. Electrons exist only at fixed levels of potential energy called electron shells.

(a) A ball bouncing down a flight of stairs can come to rest only on each step, not between steps. Similarly, an electron can exist only at certain energy levels, not between levels.



(b) An electron can move from one shell to another only if the energy it gains or loses is exactly equal to the difference in energy between the energy levels of the two shells. Arrows in this model indicate some of the stepwise changes in potential energy that are possible.



Figure Walkthrough

SCIENTIFIC SKILLS EXERCISE

Calibrating a Standard Radioactive Isotope Decay Curve and Interpreting Data

How Long Might Neanderthals Have Co-Existed with Modern Humans (*Homo sapiens*)?

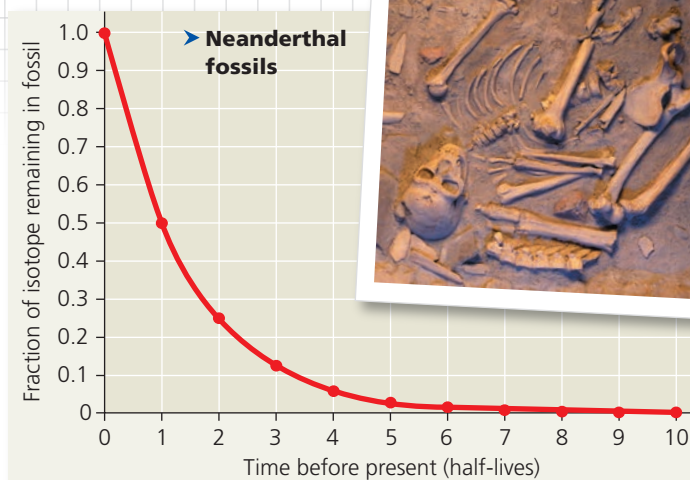
Neanderthals (*Homo neanderthalensis*) were living in Europe by 350,000 years ago and may have coexisted with early *Homo sapiens* in parts of Eurasia for hundreds or thousands of years before Neanderthals became extinct. Researchers sought to more accurately determine the extent of their overlap by pinning down the latest date Neanderthals still lived in the area. They used carbon-14 dating to determine the age of a Neanderthal fossil from the most recent (uppermost) archeological layer containing Neanderthal bones. In this exercise you will calibrate a standard carbon-14 decay curve and use it to determine the age of this Neanderthal fossil. The age will help you approximate the last time the two species may have coexisted at the site where this fossil was collected.

How the Experiment Was Done Carbon-14 (^{14}C) is a radioactive isotope of carbon that decays to ^{14}N at a constant rate. ^{14}C is present in the atmosphere in small amounts at a constant ratio with both ^{13}C and ^{12}C , two other isotopes of carbon. When carbon is taken up from the atmosphere by a plant during photosynthesis, ^{12}C , ^{13}C , and ^{14}C isotopes are incorporated into the plant in the same proportions in which they were present in the atmosphere. These proportions remain the same in the tissues of an animal that eats the plant. While an organism is alive, the ^{14}C in its body constantly decays to ^{14}N but is constantly replaced by new carbon from the environment. Once an organism dies, it stops taking in new ^{14}C but the ^{14}C in its tissues continues to decay, while the ^{12}C in its tissues remains the same because it is not radioactive and does not decay. Thus, scientists can calculate how long the pool of original ^{14}C has been decaying in a fossil by measuring the ratio of ^{14}C to ^{12}C and comparing it to the ratio of ^{14}C to ^{12}C present originally in the atmosphere. The fraction of ^{14}C in a fossil compared to the original fraction of ^{14}C can be converted to years because we know that the half-life of ^{14}C is 5,730 years—in other words, half of the ^{14}C in a fossil decays every 5,730 years.

Data from the Experiment The researchers found that the Neanderthal fossil had approximately 0.0078 (or, in scientific notation, 7.8×10^{-3}) as much ^{14}C as the atmosphere. The following questions will guide you through translating this fraction into the age of the fossil.

INTERPRET THE DATA

1. A standard curve of radioactive isotope decay is shown at the top of the right column. The graph line shows the fraction of the radioactive isotope over time (before present) in units of half-lives. Recall that a half-life is the amount of time it takes for half of the radioactive isotope to decay. Labeling each data point with the corresponding fractions will help orient you to this graph. Draw an arrow to the data point for half-life = 1 and write the



Data from R. Pinhasi et al., Revised age of late Neanderthal occupation and the end of the Middle Paleolithic in the northern Caucasus, *Proceedings of the National Academy of Sciences USA* 147:8611–8616 (2011). doi 10.1073/pnas.1018938108

fraction of ^{14}C that will remain after one half-life. Calculate the fraction of ^{14}C remaining at each half-life and write the fractions on the graph near arrows pointing to the data points. Convert each fraction to a decimal number and round off to a maximum of three significant digits (zeros at the beginning of the number do not count as significant digits). Also write each decimal number in scientific notation.

2. Recall that ^{14}C has a half-life of 5,730 years. To calibrate the x-axis for ^{14}C decay, write the time before present in years below each half-life.
3. The researchers found that the Neanderthal fossil had approximately 0.0078 as much ^{14}C as found originally in the atmosphere. (a) Using the numbers on your graph, determine how many half-lives have passed since the Neanderthal died. (b) Using your ^{14}C calibration on the x-axis, what is the approximate age of the Neanderthal fossil in years (round off to the nearest thousand)? (c) Approximately when did Neanderthals become extinct according to this study? (d) The researchers cite evidence that modern humans (*H. sapiens*) became established in the same region as the last Neanderthals approximately 39,000–42,000 years ago. What does this suggest about the overlap of Neanderthals and modern humans?
4. Carbon-14 dating works for fossils up to about 75,000 years old; fossils older than that contain too little ^{14}C to be detected. Most dinosaurs went extinct 65.5 million years ago. (a) Can ^{14}C be used to date dinosaur bones? Explain. (b) Radioactive uranium-235 has a half-life of 704 million years. If it was incorporated into dinosaur bones, could it be used to date the dinosaur fossils? Explain.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

electrons are attracted to the positively charged nucleus. It takes work to move a given electron farther away from the nucleus, so the more distant an electron is from the nucleus, the greater its potential energy. Unlike the continuous flow of water downhill, changes in the potential energy of electrons can occur only in steps of fixed amounts. An electron having a

certain amount of energy is something like a ball on a staircase (Figure 2.6a). The ball can have different amounts of potential energy, depending on which step it is on, but it cannot spend much time between the steps. Similarly, an electron's potential energy is determined by its energy level. An electron can exist only at certain energy levels, not between them.

An electron's energy level is correlated with its average distance from the nucleus. Electrons are found in different **electron shells**, each with a characteristic average distance and energy level. In diagrams, shells can be represented by concentric circles, as they are in Figure 2.6b. The first shell is closest to the nucleus, and electrons in this shell have the lowest potential energy. Electrons in the second shell have more energy, and electrons in the third shell even more energy. An electron can move from one shell to another, but only by absorbing or losing an amount of energy equal to the difference in potential energy between its position in the old shell and that in the new shell. When an electron absorbs energy, it moves to a shell farther out from the nucleus. For example, light energy can excite an electron to a higher energy level. (Indeed, this is the first step taken when plants harness the energy of sunlight for photosynthesis, the process that produces food from carbon dioxide and water. You'll learn more about photosynthesis in Chapter 11.) When an electron loses energy, it "falls back" to a shell closer to the nucleus, and the lost energy is usually released to the environment as heat. For example, sunlight

excites electrons in the surface of a car to higher energy levels. When the electrons fall back to their original levels, the car's surface heats up. This thermal energy can be transferred to the air or to your hand if you touch the car.

Electron Distribution and Chemical Properties

The chemical behavior of an atom is determined by the distribution of electrons in the atom's electron shells. Beginning with hydrogen, the simplest atom, we can imagine building the atoms of the other elements by adding 1 proton and 1 electron at a time (along with an appropriate number of neutrons).

Figure 2.7, a modified version of what is called the *periodic table of the elements*, shows this distribution of electrons for the first 18 elements, from hydrogen (${}_1\text{H}$) to argon (${}_{18}\text{Ar}$). The elements are arranged in three rows, or *periods*, corresponding to the number of electron shells in their atoms. The left-to-right sequence of elements in each row corresponds to the sequential addition of electrons and protons. (See Appendix B for the complete periodic table.)

▼ Figure 2.7 Electron distribution diagrams for the first 18 elements in the periodic table.

In a standard periodic table (see Appendix B), information for each element is presented as shown for helium in the inset. In the diagrams in this table, electrons are represented as yellow dots and electron shells as concentric circles. These diagrams are a convenient way to picture the distribution of an atom's electrons among its electron shells, but these simplified models do not accurately represent the shape of the atom or the location of its electrons. The elements are arranged in rows, each representing the filling of an electron shell. As electrons are added, they occupy the lowest available shell.

First shell	2 He 4.003 Atomic number Element symbol Atomic mass Electron distribution diagram							
	Hydrogen ${}_1\text{H}$	Helium ${}_2\text{He}$						
Second shell	Lithium ${}_3\text{Li}$	Beryllium ${}_4\text{Be}$	Boron ${}_5\text{B}$	Carbon ${}_6\text{C}$	Nitrogen ${}_7\text{N}$	Oxygen ${}_8\text{O}$	Fluorine ${}_9\text{F}$	Neon ${}_{10}\text{Ne}$
Third shell	Sodium ${}_{11}\text{Na}$	Magnesium ${}_{12}\text{Mg}$	Aluminum ${}_{13}\text{Al}$	Silicon ${}_{14}\text{Si}$	Phosphorus ${}_{15}\text{P}$	Sulfur ${}_{16}\text{S}$	Chlorine ${}_{17}\text{Cl}$	Argon ${}_{18}\text{Ar}$

VISUAL SKILLS ► Looking at the depictions of atoms in this chart, what is the atomic number of magnesium? How many protons and electrons does it have? How many electron shells? How many valence electrons?



Animation: Electron Distribution Diagrams

Hydrogen's 1 electron and helium's 2 electrons are located in the first shell. Electrons, like all matter, tend to exist in the lowest available state of potential energy. In an atom, this state is in the first shell. However, the first shell can hold no more than 2 electrons; thus, hydrogen and helium are the only elements in the first row of the table. In an atom with more than 2 electrons, the additional electrons must occupy higher shells because the first shell is full. The next element, lithium, has 3 electrons. Two of these electrons fill the first shell, while the third electron occupies the second shell. The second shell holds a maximum of 8 electrons. Neon, at the end of the second row, has 8 electrons in the second shell, giving it a total of 10 electrons.

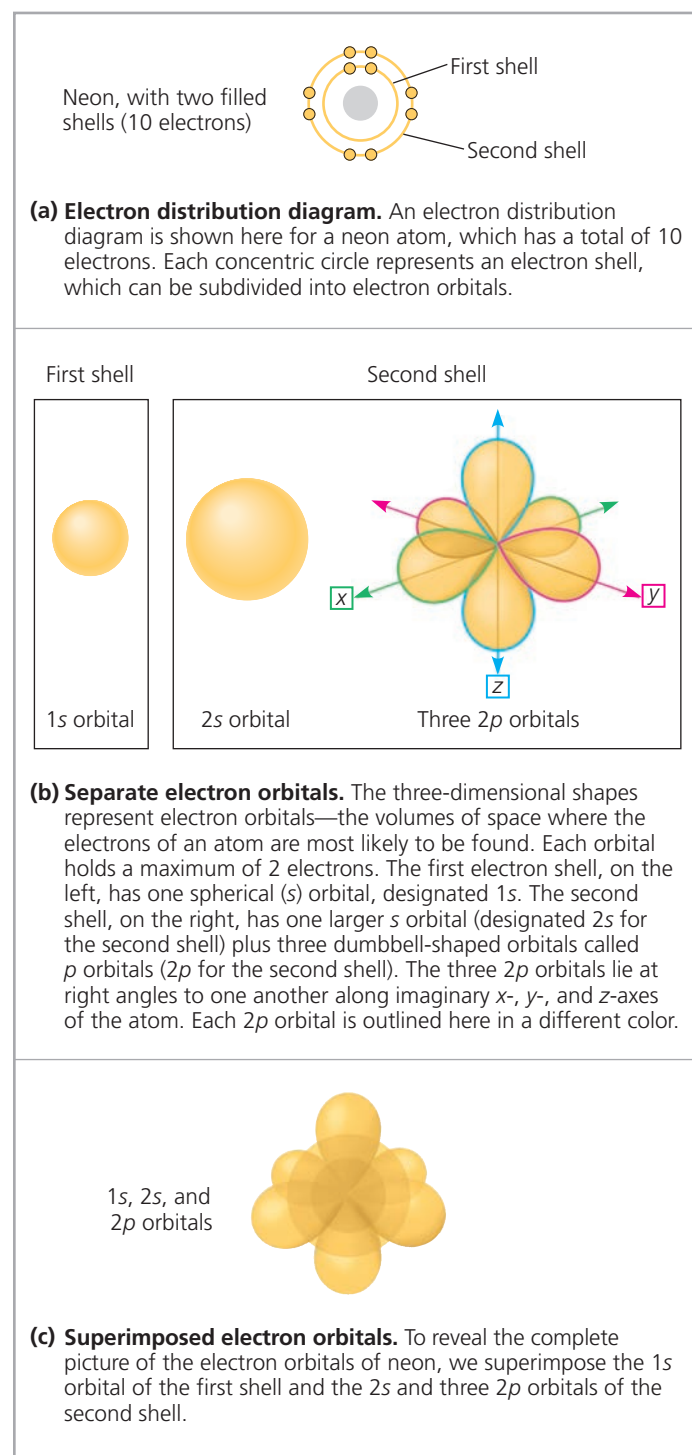
The chemical behavior of an atom depends mostly on the number of electrons in its *outermost* shell. We call those outer electrons **valence electrons** and the outermost electron shell the **valence shell**. In the case of lithium, there is only 1 valence electron, and the second shell is the valence shell. Atoms with the same number of electrons in their valence shells exhibit similar chemical behavior. For example, fluorine (F) and chlorine (Cl) both have 7 valence electrons, and both form compounds when combined with the element sodium (Na): Sodium fluoride (NaF) is commonly added to toothpaste to prevent tooth decay, and, as described earlier, NaCl is table salt (see Figure 2.2). An atom with a completed valence shell is unreactive; that is, it will not interact readily with other atoms. At the far right of the periodic table are helium, neon, and argon, the only three elements shown in Figure 2.7 that have full valence shells. These elements are said to be *inert*, meaning chemically unreactive. All the other atoms in Figure 2.7 are chemically reactive because they have incomplete valence shells.

Electron Orbitals

In the early 1900s, the electron shells of an atom were visualized as concentric paths of electrons orbiting the nucleus, somewhat like planets orbiting the sun. It is still convenient to use two-dimensional concentric-circle diagrams, as in Figure 2.7, to symbolize three-dimensional electron shells. However, you need to remember that each concentric circle represents only the *average* distance between an electron in that shell and the nucleus. Accordingly, the concentric-circle diagrams do not give a real picture of an atom. In reality, we can never know the exact location of an electron. What we can do instead is describe the space in which an electron spends most of its time. The three-dimensional space where an electron is found 90% of the time is called an **orbital**.

Each electron shell contains electrons at a particular energy level, distributed among a specific number of orbitals of distinctive shapes and orientations. **Figure 2.8** shows the orbitals of neon as an example, with its electron distribution diagram for reference. You can think of an orbital as a component of an electron shell. The first electron shell has only one spherical *s* orbital (called 1*s*), but the second shell has

▼ **Figure 2.8 Electron orbitals.**



four orbitals: one large spherical *s* orbital (called 2*s*) and three dumbbell-shaped *p* orbitals (called 2*p* orbitals). (The third shell and other higher electron shells also have *s* and *p* orbitals, as well as orbitals of more complex shapes.)

No more than 2 electrons can occupy a single orbital. The first electron shell can therefore accommodate up to 2 electrons in its *s* orbital. The lone electron of a hydrogen atom occupies the 1*s* orbital, as do the 2 electrons of a helium atom. The four orbitals of the second electron shell can hold up to 8 electrons,

2 in each orbital. Electrons in each of the four orbitals in the second shell have nearly the same energy, but they move in different volumes of space.

The reactivity of an atom arises from the presence of unpaired electrons in one or more orbitals of the atom's valence shell. As you will see in the next section, atoms interact in a way that completes their valence shells. When they do so, it is the *unpaired* electrons that are involved.

CONCEPT CHECK 2.2

1. A lithium atom has 3 protons and 4 neutrons. What is its mass number?
2. A hydrogen atom has 1 proton, and the most common isotope of hydrogen has 0 neutrons. A radioactive isotope of hydrogen has 2 neutrons. Write the atomic number and mass number of this radioactive hydrogen as a chemical symbol with a subscript and superscript.
3. How many electrons does fluorine have? How many electron shells? Name the orbitals that are occupied. How many electrons are needed to fill the valence shell?
4. **VISUAL SKILLS** > In Figure 2.7, if two or more elements are in the same row, what do they have in common? If two or more elements are in the same column, what do they have in common?

For suggested answers, see Appendix A.

CONCEPT 2.3

The formation and function of molecules depend on chemical bonding between atoms

Now that we have looked at the structure of atoms, we can move up the hierarchy of organization and see how atoms combine to form molecules and ionic compounds. Atoms with incomplete valence shells can interact with certain other atoms in such a way that each partner atom completes its valence shell: The atoms either share or transfer valence electrons. These interactions usually result in atoms staying close together, held by attractions called **chemical bonds**. The strongest kinds of chemical bonds are covalent bonds and ionic bonds in dry ionic compounds. (Ionic bonds in aqueous, or water-based, solutions are weak interactions, as we will see later.)

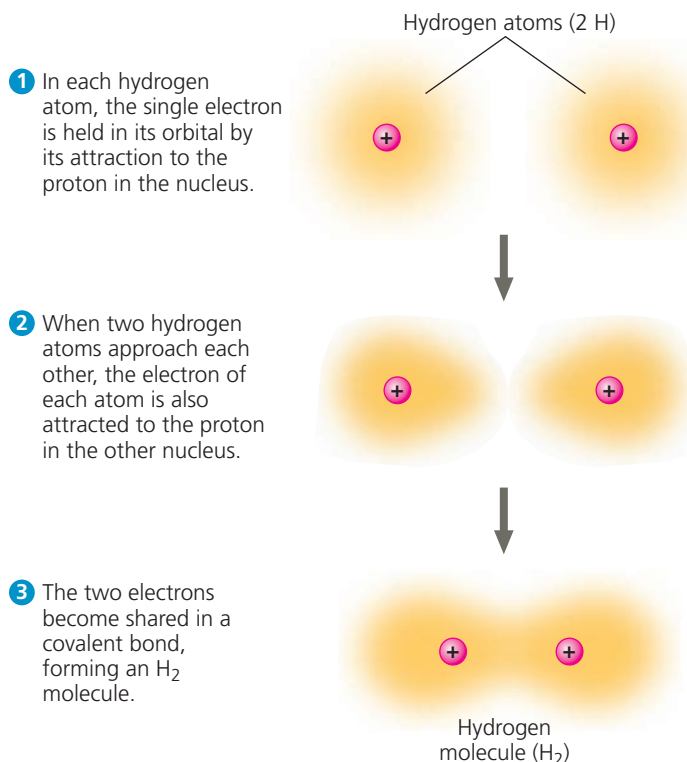


Animation: Introduction to Chemical Bonds

Covalent Bonds

A **covalent bond** is the sharing of a pair of valence electrons by two atoms. For example, let's consider what happens when two hydrogen atoms approach each other. Recall that hydrogen has 1 valence electron in the first shell, but the shell's capacity is 2 electrons. When the two hydrogen atoms come close enough for their 1s orbitals to overlap, they can share their electrons (**Figure 2.9**). Each hydrogen atom is now associated with 2 electrons in what amounts to a completed valence shell. Two

▼ **Figure 2.9** Formation of a covalent bond.



or more atoms held together by covalent bonds constitute a **molecule**, in this case a hydrogen molecule.

Figure 2.10a shows several ways of representing a hydrogen molecule. Its *molecular formula*, H_2 , simply indicates that the molecule consists of two atoms of hydrogen. Electron sharing can be depicted by an electron distribution diagram or by a *Lewis dot structure*, in which element symbols are surrounded by dots that represent the valence electrons ($H:H$). We can also use a *structural formula*, $H-H$, where the line represents a **single bond**, a pair of shared electrons. A *space-filling model* comes closest to representing the actual shape of the molecule. (You may also be familiar with ball-and-stick models, which are shown in Figure 2.15.)

Oxygen has 6 electrons in its second electron shell and therefore needs 2 more electrons to complete its valence shell. Two oxygen atoms form a molecule by sharing *two* pairs of valence electrons (**Figure 2.10b**). The atoms are thus joined by what is called a **double bond** ($O=O$).

Each atom that can share valence electrons has a bonding capacity corresponding to the number of covalent bonds the atom can form. When the bonds form, they give the atom a full complement of electrons in the valence shell. The bonding capacity of oxygen, for example, is 2. This bonding capacity is called the atom's **valence** and usually equals the number of unpaired electrons required to complete the atom's outermost (valence) shell. See if you can determine the valences of hydrogen, oxygen, nitrogen, and carbon by studying the electron distribution diagrams in Figure 2.7. You can see that the valence of hydrogen is 1; oxygen, 2; nitrogen, 3; and carbon, 4. The situation is more complicated

▼ **Figure 2.10 Covalent bonding in four molecules.** The number of electrons required to complete an atom's valence shell generally determines how many covalent bonds that atom will form. This figure shows several ways of indicating covalent bonds.

Name and Molecular Formula	Electron Distribution Diagram	Lewis Dot Structure and Structural Formula	Space-Filling Model
(a) Hydrogen (H₂). Two hydrogen atoms share one pair of electrons, forming a single bond.		H : H H — H	
(b) Oxygen (O₂). Two oxygen atoms share two pairs of electrons, forming a double bond.		::O::O: O = O	
(c) Water (H₂O). Two hydrogen atoms and one oxygen atom are joined by single bonds, forming a molecule of water.		::O::H H O — H H	
(d) Methane (CH₄). Four hydrogen atoms can satisfy the valence of one carbon atom, forming methane.		H H : C : H H H — C — H H	

Animation: Covalent Bonds

for phosphorus, in the third row of the periodic table, which can have a valence of 3 or 5 depending on the combination of single and double bonds it makes.

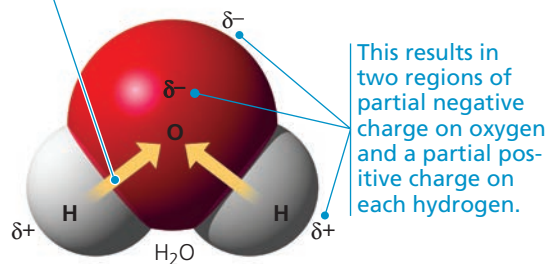
The molecules H₂ and O₂ are pure elements rather than compounds because a compound is a combination of two or more *different* elements. Water, with the molecular formula H₂O, is a compound. Two atoms of hydrogen are needed to satisfy the valence of one oxygen atom. **Figure 2.10c** shows the structure of a water molecule. (Water is so important to life that Chapter 3 is devoted entirely to its structure and behavior.)

Methane, the main component of natural gas, is a compound with the molecular formula CH₄. It takes four hydrogen atoms, each with a valence of 1, to complement one atom of carbon, with its valence of 4 (**Figure 2.10d**). (We will look at many other compounds of carbon in Chapter 4.)

Atoms in a molecule attract shared bonding electrons to varying degrees, depending on the element. The attraction of a particular atom for the electrons of a covalent bond is called its

▼ **Figure 2.11 Polar covalent bonds in a water molecule.**

Because oxygen (O) is more electronegative than hydrogen (H), shared electrons are pulled more toward oxygen.



Animation: Nonpolar and Polar Molecules

electronegativity. The more electronegative an atom is, the more strongly it pulls shared electrons toward itself. In a covalent bond between two atoms of the same element, the electrons are shared equally because the two atoms have the same electronegativity—the tug-of-war is at a standoff. Such a bond is called a **nonpolar covalent bond**. For example, the single bond of H₂ is nonpolar, as is the double bond of O₂. However, when an atom is bonded to a more electronegative atom, the electrons of the bond are not shared equally. This type of bond is called a **polar covalent bond**. Such bonds vary in their polarity, depending on the relative electronegativity of the two atoms. For example, the bonds between the oxygen and hydrogen atoms of a water molecule are quite polar (**Figure 2.11**).

Oxygen is one of the most electronegative elements, attracting shared electrons much more strongly than hydrogen does. In a covalent bond between oxygen and hydrogen, the electrons spend more time near the oxygen nucleus than near the hydrogen nucleus. Because electrons have a negative charge and are pulled toward oxygen in a water molecule, the oxygen atom has two regions of partial negative charge (each indicated by the Greek letter δ with a minus sign, δ^- , “delta minus”), and each hydrogen atom has a partial positive charge (δ^+ , “delta plus”). In contrast, the individual bonds of methane (CH₄) are much less polar because the electronegativities of carbon and hydrogen are similar.

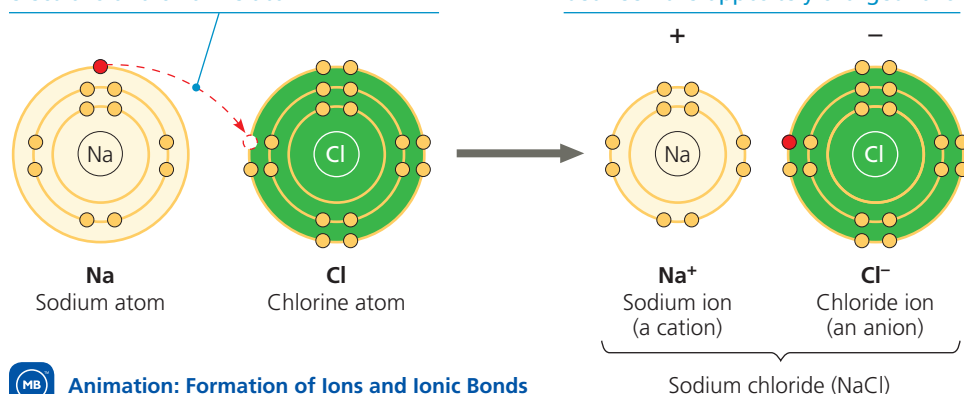
Ionic Bonds

In some cases, two atoms are so unequal in their attraction for valence electrons that the more electronegative atom strips an electron completely away from its partner. The two resulting oppositely charged atoms (or molecules) are called **ions**. A positively charged ion is called a **cation**, while a negatively charged ion is called an **anion**. Because of their opposite charges, cations and anions attract each other; this attraction is called an **ionic bond**. Note that the transfer of an electron is not, by itself, the formation of a bond; rather, it allows a bond to form because it results in two ions of opposite charge. Any two ions of opposite charge can form an ionic bond. The ions do not need to have acquired their charge by an electron transfer with each other.

▼ **Figure 2.12 Electron transfer and ionic bonding.** The attraction between oppositely charged atoms, or ions, is an ionic bond. An ionic bond can form between any two oppositely charged ions, even if they have not been formed by transfer of an electron from one to the other.

❶ The lone valence electron of a sodium atom is transferred to join the 7 valence electrons of a chlorine atom.

❷ Each resulting ion has a completed valence shell. An ionic bond can form between the oppositely charged ions.

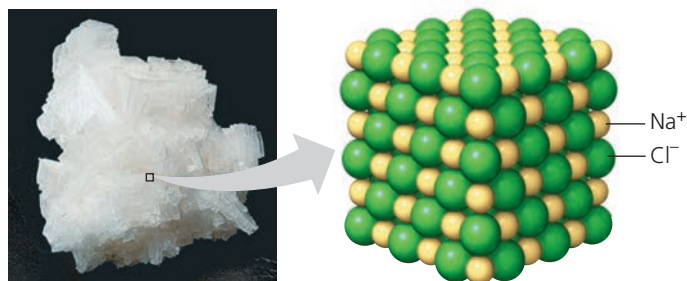


MB Animation: Formation of Ions and Ionic Bonds

This is what happens when an atom of sodium ($_{11}\text{Na}$) encounters an atom of chlorine ($_{17}\text{Cl}$) (**Figure 2.12**). A sodium atom has a total of 11 electrons, with its single valence electron in the third electron shell. A chlorine atom has a total of 17 electrons, with 7 electrons in its valence shell. When these two atoms meet, the lone valence electron of sodium is transferred to the chlorine atom, and both atoms end up with their valence shells complete. (Because sodium no longer has an electron in the third shell, the second shell is now the valence shell.) The electron transfer between the two atoms moves one unit of negative charge from sodium to chlorine. Sodium, now with 11 protons but only 10 electrons, has a net electrical charge of $1+$; the sodium atom has become a cation. Conversely, the chlorine atom, having gained an extra electron, now has 17 protons and 18 electrons, giving it a net electrical charge of $1-$; it has become a chloride ion—an anion.

Compounds formed by ionic bonds are called **ionic compounds**, or **salts**. We know the ionic compound sodium chloride (NaCl) as table salt (**Figure 2.13**). Salts are often found in nature as crystals of various sizes and shapes. Each salt crystal is an aggregate of vast numbers of cations and anions bonded by their electrical attraction and arranged in a three-dimensional lattice. Unlike a covalent compound,

▼ **Figure 2.13 A sodium chloride (NaCl) crystal.** The sodium ions (Na^+) and chloride ions (Cl^-) are held together by ionic bonds. The formula NaCl tells us that the ratio of Na^+ to Cl^- is $1:1$.



which consists of molecules having a definite size and number of atoms, an ionic compound does not consist of molecules. The formula for an ionic compound, such as NaCl , indicates only the ratio of elements in a crystal of the salt. “ NaCl ” by itself is not a molecule.

Not all salts have equal numbers of cations and anions. For example, the ionic compound magnesium chloride (MgCl_2) has two chloride ions for each magnesium ion. Magnesium ($_{12}\text{Mg}$) must lose 2 outer electrons if the atom is to have a complete valence shell, so it has a tendency to become a cation with a net charge of $2+$ (Mg^{2+}). One magnesium cation can therefore form ionic bonds with two chloride anions (Cl^-).

The term *ion* also applies to entire molecules that are electrically charged. In the salt ammonium chloride (NH_4Cl), for instance, the anion is a single chloride ion (Cl^-), but the cation is ammonium (NH_4^+), a nitrogen atom covalently bonded to four hydrogen atoms. The whole ammonium ion has an electrical charge of $1+$ because it has given up 1 electron and thus is 1 electron short.

Environment affects the strength of ionic bonds. In a dry salt crystal, the bonds are so strong that it takes a hammer and chisel to break enough of them to crack the crystal in two. If the same salt crystal is dissolved in water, however, the ionic bonds are much weaker because each ion is partially shielded by its interactions with water molecules. Most drugs are manufactured as salts because they are quite stable when dry but can dissociate (come apart) easily in water. (In Concept 3.2, you will learn how water dissolves salts.)

Weak Chemical Interactions

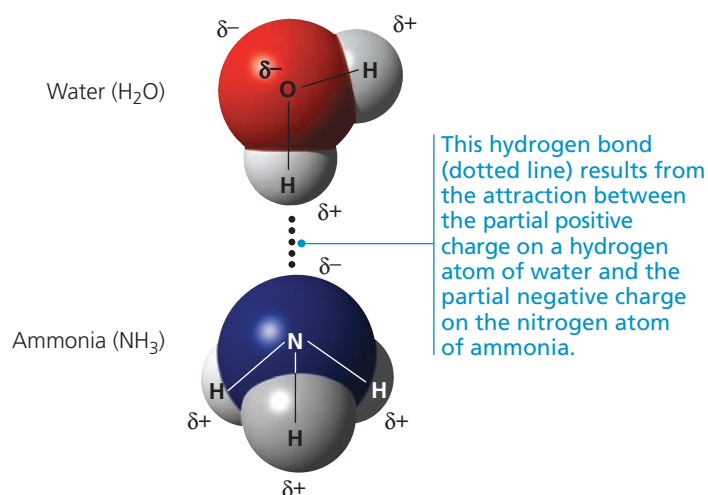
In organisms, most of the strongest chemical bonds are covalent bonds, which link atoms to form a cell’s molecules. But weaker interactions within and between molecules are also indispensable, contributing greatly to the emergent properties of life. Many large biological molecules are held in their functional form by weak interactions. In addition, when two molecules in the cell make contact, they may adhere temporarily by weak interactions. The reversibility of weak interactions can be an advantage: Two molecules can come together, affect one another in some way, and then separate.

Several types of weak chemical interactions are important in organisms. One is the ionic bond as it exists between ions dissociated in water, which we just discussed. Hydrogen bonds and van der Waals interactions are also crucial to life.

Hydrogen Bonds

Among weak chemical interactions, hydrogen bonds are so central to the chemistry of life that they deserve special

▼ **Figure 2.14 A hydrogen bond.**



DRAW IT ► Draw one water molecule surrounded by four other water molecules, arranged so that they can make hydrogen bonds with each other. Use simple outlines of space-filling models. Draw the partial charges on the water molecules and use dots for the hydrogen bonds.

MB Animation: Hydrogen Bonds

attention. When a hydrogen atom is covalently bonded to an electronegative atom, the hydrogen atom has a partial positive charge that allows it to be attracted to a different electronegative atom nearby. This attraction between a hydrogen and an electronegative atom is called a **hydrogen bond**. In living cells, the electronegative partners are usually oxygen or nitrogen atoms. Refer to **Figure 2.14** to examine the simple case of hydrogen bonding between water (H_2O) and ammonia (NH_3).

Van der Waals Interactions

Even a molecule with nonpolar covalent bonds may have positively and negatively charged regions. Electrons are not always evenly distributed; at any instant, they may accumulate by chance in one part of a molecule or another. The results are ever-changing regions of positive and negative charge that enable all atoms and molecules to stick to one another. These **van der Waals interactions** are individually weak and occur only when atoms and molecules are very close together. When many such interactions occur simultaneously, however, they can be powerful: Van der Waals interactions allow the gecko lizard shown here to walk straight up a wall! The anatomy of the gecko's foot—including many minuscule hairlike projections from the toes and strong tendons underlying the skin—strikes a balance between maximum surface

contact with the wall and necessary stiffness of the foot. The van der Waals interactions between the foot molecules and the molecules of

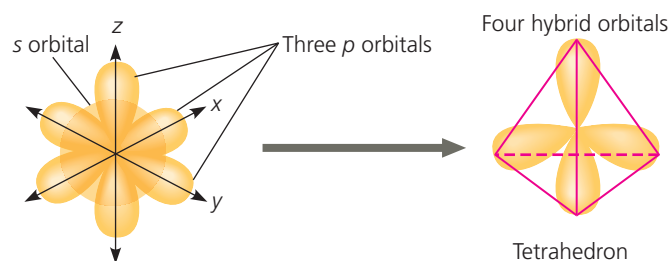
the wall's surface are so numerous that despite their individual weakness, together they can support the gecko's body weight. This discovery has inspired development of an artificial adhesive called Geckskin: A patch the size of an index card can hold a 700-pound weight to a wall!

Van der Waals interactions, hydrogen bonds, ionic bonds in water, and other weak interactions may form not only between molecules but also between parts of a large molecule, such as a protein. The cumulative effect of weak interactions is to reinforce the three-dimensional shape of the molecule. (You will learn more about the very important biological roles of weak interactions in Chapter 5.)

Molecular Shape and Function

A molecule has a characteristic size and shape, which are key to its function in the living cell. A molecule consisting of two atoms, such as H_2 or O_2 , is always linear, but most molecules with more than two atoms have more complicated shapes. These shapes are determined by the positions of the atoms' orbitals (**Figure 2.15**). When an atom forms covalent bonds,

▼ **Figure 2.15 Molecular shapes due to hybrid orbitals.**



(a) Hybridization of orbitals. The single s and three p orbitals of a valence shell involved in covalent bonding combine to form four teardrop-shaped hybrid orbitals. These orbitals extend to the four corners of an imaginary tetrahedron (outlined in pink).

Space-Filling Model	Ball-and-Stick Model	Hybrid-Orbital Model (with ball-and-stick model superimposed)
Water (H_2O)	104.5°	Unbonded electron pairs
Methane (CH_4)		

(b) Molecular-shape models. Three models representing molecular shape are shown for water and methane. The positions of the hybrid orbitals determine the shapes of the molecules.

the orbitals in its valence shell undergo rearrangement. For atoms with valence electrons in both *s* and *p* orbitals (review Figure 2.8), the single *s* and three *p* orbitals form four new hybrid orbitals shaped like identical teardrops extending from the region of the atomic nucleus, as shown in Figure 2.15a. If we connect the larger ends of the teardrops with lines, we have the outline of a geometric shape called a tetrahedron, a pyramid with a triangular base.

For water molecules (H_2O), two of the hybrid orbitals in the oxygen's valence shell are shared with hydrogens (see Figure 2.15b). The result is a molecule shaped roughly like a V, with its two covalent bonds at an angle of 104.5° .

The methane molecule (CH_4) has the shape of a completed tetrahedron because all four hybrid orbitals of the carbon atom are shared with hydrogen atoms (see Figure 2.15b). The carbon nucleus is at the center, with its four covalent bonds radiating to hydrogen nuclei at the corners of the tetrahedron. Larger molecules containing multiple carbon atoms, including many of the molecules that make up living matter, have more complex overall shapes. However, the tetrahedral shape of a carbon atom bonded to four other atoms is often a repeating motif within such molecules.

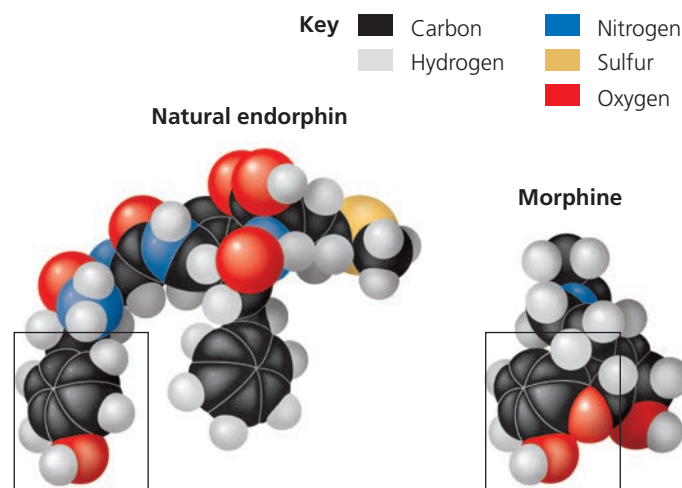
Molecular shape is crucial: It determines how biological molecules recognize and respond to one another with specificity. Biological molecules often bind temporarily to each other by forming weak interactions, but only if their shapes are complementary. Consider the effects of opiates, drugs such as morphine and heroin derived from opium. Opiates relieve pain and alter mood by weakly binding to specific receptor molecules on the surfaces of brain cells. Why would brain cells carry receptors for opiates, compounds that are not made by the body? In 1975, the discovery of endorphins answered this question. Endorphins are signaling molecules made by the pituitary gland that bind to the receptors, relieving pain and producing euphoria during times of stress, such as intense exercise. Opiates have shapes similar to endorphins and mimic them by binding to endorphin receptors in the brain. That is why opiates and endorphins have similar effects (Figure 2.16). The role of molecular shape in brain chemistry illustrates how biological organization leads to a match between structure and function, one of biology's unifying themes.

CONCEPT CHECK 2.3

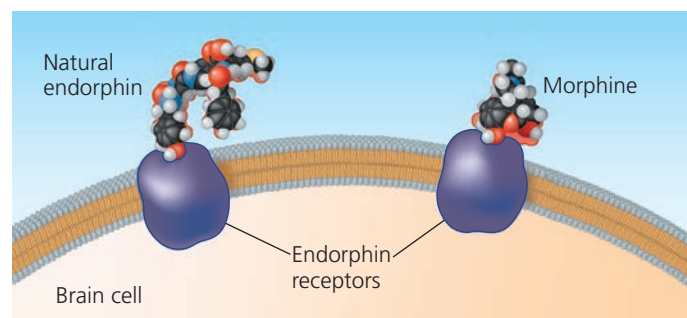
1. Why does the structure $\text{H}-\text{C}=\text{C}-\text{H}$ fail to make sense chemically?
2. Which bond is more polar: the $\text{O}-\text{H}$ bond in H_2O or the $\text{C}-\text{H}$ bond in CH_4 ?
3. **WHAT IF? >** If you were a pharmaceutical researcher, why would you want to learn the three-dimensional shapes of naturally occurring signaling molecules?

For suggested answers, see Appendix A.

▼ **Figure 2.16 A molecular mimic.** Morphine affects pain perception and emotional state by mimicking the brain's natural endorphins.



(a) Structures of endorphin and morphine. The boxed portion of the endorphin molecule (left) binds to receptor molecules on target cells in the brain. The boxed portion of the morphine molecule (right) is a close match.



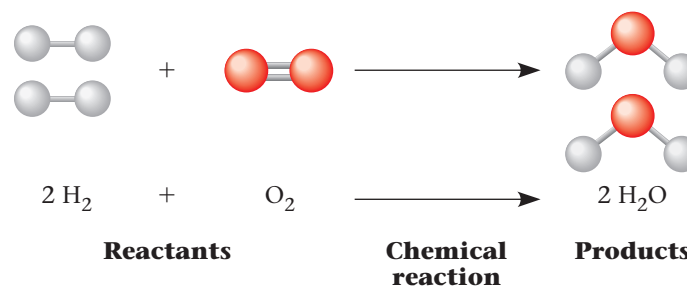
(b) Binding to endorphin receptors. Both endorphin and morphine can bind to endorphin receptors on the surface of a brain cell.

MB Interview with Candace Pert: Discovering opiate receptors in the brain

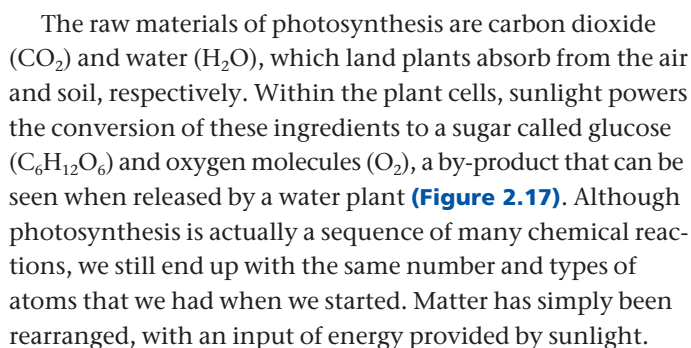
CONCEPT 2.4

Chemical reactions make and break chemical bonds

The making and breaking of chemical bonds, leading to changes in the composition of matter, are called **chemical reactions**. An example is the reaction between hydrogen and oxygen molecules that forms water:



Photosynthesis, which takes place within the cells of green plant tissues, is an important biological example of how chemical reactions rearrange matter. Humans and other animals ultimately depend on photosynthesis for food and oxygen, and this process is at the foundation of almost all ecosystems. The following summarizes the process of photosynthesis:


$$3 \text{H}_2 + \text{N}_2 \rightleftharpoons 2 \text{NH}_3$$

One of the factors affecting the rate of a reaction is the concentration of reactants. The greater the concentration of reactant molecules, the more frequently they collide with one another and have an opportunity to react and form products.

A photograph of a green, curly, submerged plant stem. The stem is bright green and has several dark green, curled leaves. Small, clear bubbles are visible on the stem. Two labels with arrows point to specific parts: 'Leaf' points to a dark green leaf, and 'Bubbles of O₂' points to a cluster of small bubbles on the stem.

The same holds true for products. As products accumulate, collisions resulting in the reverse reaction become more frequent. Eventually, the forward and reverse reactions occur at the same rate, and the relative concentrations of products and reactants stop changing. The point at which the reactions offset one another exactly is called **chemical equilibrium**. This is a dynamic equilibrium; reactions are still going on in both directions, but with no net effect on the concentrations of reactants and products. Equilibrium does *not* mean that the reactants and products are equal in concentration, but only that their concentrations have stabilized at a particular ratio. The reaction involving ammonia reaches equilibrium when ammonia decomposes as rapidly as it forms. In some chemical reactions, the equilibrium point may lie so far to the right that these reactions go essentially to completion; that is, virtually all the reactants are converted to products.

We will return to the subject of chemical reactions after more detailed study of the various types of molecules that are important to life. In the next chapter, we focus on water, the substance in which all the chemical processes of organisms occur.

1. **MAKE CONNECTIONS** ➤ Consider the reaction between hydrogen and oxygen that forms water, shown with ball-and-stick models at the beginning of Concept 2.4. After studying Figure 2.10, draw and label the Lewis dot structures representing this reaction.
2. Which type of chemical reaction, if any, occurs faster at equilibrium: the formation of products from reactants or that of reactants from products?
3. **WHAT IF?** ➤ Write an equation that uses the products of photosynthesis as reactants and the reactants of photosynthesis as products. Add energy as another product. This new equation describes a process that occurs in your cells. Describe this equation in words. How does this equation relate to breathing?

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2 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 2.1

Matter consists of chemical elements in pure form and in combinations called compounds (pp. 77–78)



VOCAB
SELF-QUIZ
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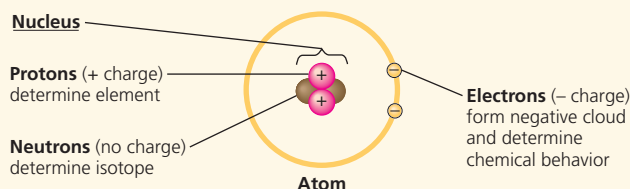
- **Elements** cannot be broken down chemically to other substances. A **compound** contains two or more different elements in a fixed ratio. Oxygen, carbon, hydrogen, and nitrogen make up approximately 96% of living matter.

? Compare an element and a compound.

CONCEPT 2.2

An element's properties depend on the structure of its atoms (pp. 78–84)

- An **atom**, the smallest unit of an element, has the following components:



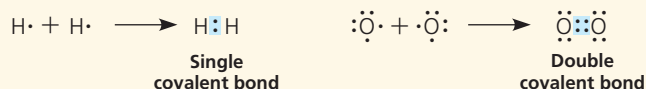
- An electrically neutral atom has equal numbers of electrons and protons; the number of protons determines the **atomic number**. The **atomic mass** is measured in **daltons** and is roughly equal to the **mass number**, the sum of protons plus neutrons. **Isotopes** of an element differ from each other in neutron number and therefore mass. Unstable isotopes give off particles and energy as radioactivity.
- In an atom, electrons occupy specific **electron shells**; the electrons in a shell have a characteristic energy level. Electron distribution in shells determines the chemical behavior of an atom. An atom that has an incomplete outer shell, the **valence shell**, is reactive.
- Electrons exist in **orbitals**, three-dimensional spaces with specific shapes that are components of electron shells.

DRAW IT ➤ Draw the electron distribution diagrams for neon ($_{10}\text{Ne}$) and argon ($_{18}\text{Ar}$). Use these diagrams to explain why these elements are chemically unreactive.

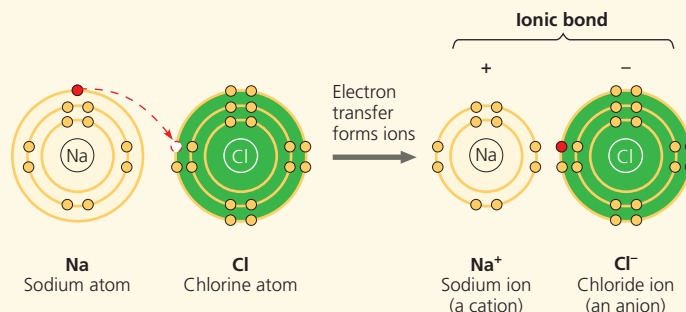
CONCEPT 2.3

The formation and function of molecules depend on chemical bonding between atoms (pp. 84–88)

- **Chemical bonds** form when atoms interact and complete their valence shells. **Covalent bonds** form when pairs of electrons are shared:



- **Molecules** consist of two or more covalently bonded atoms. The attraction of an atom for the electrons of a covalent bond is its **electronegativity**. If both atoms are the same, they have the same electronegativity and share a **nonpolar covalent bond**. Electrons of a **polar covalent bond** are pulled closer to the more electronegative atom, such as the oxygen in H_2O .
- An **ion** forms when an atom or molecule gains or loses an electron and becomes charged. An **ionic bond** is the attraction between two oppositely charged ions:



- Weak interactions reinforce the shapes of large molecules and help molecules adhere to each other. A **hydrogen bond** is an attraction between a hydrogen atom carrying a partial positive charge (δ^+) and an electronegative atom carrying a partial negative charge (δ^-). **Van der Waals interactions** occur between transiently positive and negative regions of molecules.
- A molecule's shape is determined by the positions of its atoms' valence orbitals. Covalent bonds result in hybrid orbitals, which are responsible for the shapes of H_2O , CH_4 , and many more complex biological molecules. Molecular shape is usually the basis for the recognition of one biological molecule by another.

? In terms of electron sharing between atoms, compare nonpolar covalent bonds, polar covalent bonds, and the formation of ions.

CONCEPT 2.4

Chemical reactions make and break chemical bonds (pp. 88–89)

- **Chemical reactions** change **reactants** into **products** while conserving matter. All chemical reactions are theoretically reversible. **Chemical equilibrium** is reached when the forward and reverse reaction rates are equal.

? What would happen to the concentration of products if more reactants were added to a reaction that was in chemical equilibrium? How would this addition affect the equilibrium?

TEST YOUR UNDERSTANDING



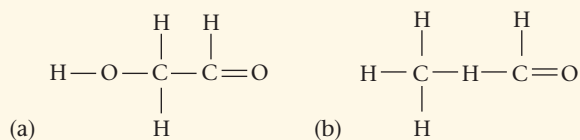
Multiple-choice Self-Quiz questions 1–8 can be found in the Study Area in MasteringBiology.

9. **DRAW IT** Draw Lewis dot structures for each hypothetical molecule shown below, using the correct number of valence electrons for each atom. Determine which molecule makes sense because each atom has a complete valence shell



PRACTICE
TEST
goo.gl/iAsVgL

and each bond has the correct number of electrons. Explain what makes the other molecule nonsensical, considering the number of bonds each type of atom can make.



- 10. EVOLUTION CONNECTION** Some species have become adapted to extremely harsh environments. How can such specially adapted species be used for the benefit of humans? Explain with a suitable example.

- 11. SCIENTIFIC INQUIRY** Female luna moths (*Actias luna*) attract males by emitting chemical signals that spread through the air. A male hundreds of meters away can detect these molecules and fly toward their source. The sensory organs responsible for this behavior are the comblike antennae visible in the photograph shown here. Each filament of an antenna is equipped with thousands of receptor cells that detect the sex attractant. Based on what you learned in this chapter, propose a hypothesis to account for the ability of the male moth to detect a specific molecule in the presence of many other molecules in the air. What predictions does your hypothesis make? Design an experiment to test one of these predictions.



- 12. WRITE ABOUT A THEME: ORGANIZATION** While waiting at an airport, Neil Campbell once overheard this claim: "It's paranoid and ignorant to worry about industry or agriculture contaminating the environment with their chemical wastes. After all, this stuff is just made of the same atoms that were

already present in our environment." Drawing on your knowledge of electron distribution, bonding, and emergent properties (see Concept 1.1), write a short essay (100–150 words) countering this argument.

13. SYNTHESIZE YOUR KNOWLEDGE



This bombardier beetle is spraying a boiling hot liquid that contains irritating chemicals, used as a defense mechanism against its enemies. The beetle stores two sets of chemicals separately in its glands. Using what you learned about chemistry in this chapter, propose a possible explanation for why the beetle is not harmed by the chemicals it stores and what causes the explosive discharge.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

The Chemistry of Water

3

Figure 3.1 How does life on Earth depend on the chemistry of water?

KEY CONCEPTS

- 3.1** Polar covalent bonds in water molecules result in hydrogen bonding
- 3.2** Four emergent properties of water contribute to Earth's suitability for life
- 3.3** Acidic and basic conditions affect living organisms



Black guillemots, threatened by climate change

The Molecule That Supports All of Life

Life on Earth began in water and evolved there for 3 billion years before spreading onto land. Water is the substance that makes life possible as we know it here on Earth, and possibly on other planets as well. All organisms familiar to us are made mostly of water and live in an environment dominated by water.

Three-quarters of Earth's surface is covered by water. Although most of this water is in liquid form, water is also present on Earth as a solid (ice) and a gas (water vapor). Water is the only common substance on Earth to exist in the natural environment in all three physical states of matter. Furthermore, the solid form of water floats on the liquid form, a rare property emerging from the chemistry of the water molecule.

As the Earth is warming from climate change (see Concept 1.1), the ratio of ice to liquid water is changing. Arctic sea ice and glaciers are melting, affecting life on, under, and around them. In the Arctic, warmer waters and the smaller ice pack are resulting in blooms of phytoplankton (microscopic aquatic photosynthetic organisms), seen from space as the “cloudy” seawater in **Figure 3.1**. Organisms that depend on Arctic ice, however, are suffering. For instance, a population of black guillemots in Alaska is declining due to the warming climate and reduction of Arctic sea ice.

In this chapter, you will learn how the structure of a water molecule allows it to interact with other molecules, including other water molecules. This ability leads to water's unique emergent properties that help make Earth suitable for life.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

CONCEPT 3.1

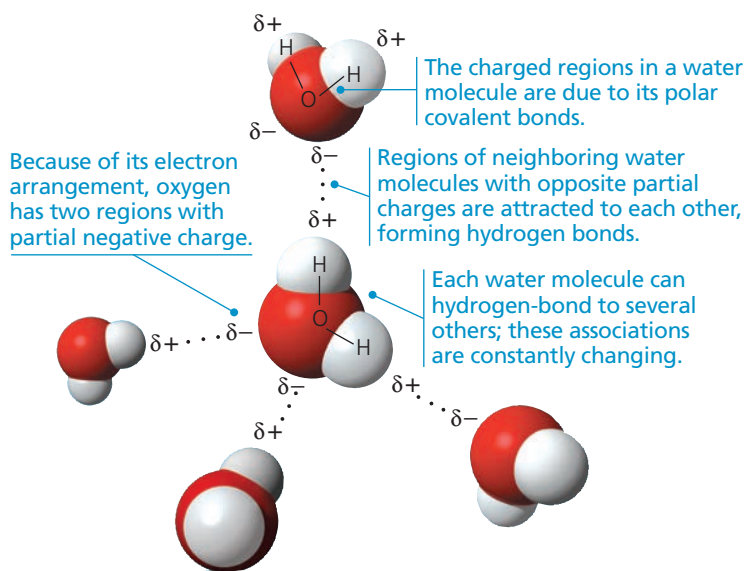
Polar covalent bonds in water molecules result in hydrogen bonding

Water is so familiar to us that it is easy to overlook its many extraordinary qualities. Following the theme of emergent properties, we can trace water's unique behavior to the structure and interactions of its molecules.

Studied on its own, the water molecule is deceptively simple. It is shaped like a wide V, with its two hydrogen atoms joined to the oxygen atom by single covalent bonds. Oxygen is more electronegative than hydrogen, so the electrons of the covalent bonds spend more time closer to oxygen than to hydrogen; these are **polar covalent bonds** (see Figure 2.11). This unequal sharing of electrons and water's V-like shape make it a **polar molecule**, meaning that its overall charge is unevenly distributed. In water, the oxygen of the molecule has two regions of partial negative charge (δ^-), and each hydrogen has a partial positive charge (δ^+).

The properties of water arise from attractions between oppositely charged atoms of different water molecules: The partially positive hydrogen of one molecule is attracted to the partially negative oxygen of a nearby molecule. The two molecules are thus held together by a hydrogen bond (Figure 3.2). When water is in its liquid form, its hydrogen bonds are very fragile, each only about 1/20 as strong as a covalent bond. The hydrogen bonds form, break, and re-form with great frequency. Each lasts only a few trillionths of a second, but the molecules are constantly forming new hydrogen bonds with a succession of partners. Therefore, at any instant, most of the water molecules

▼ **Figure 3.2** Hydrogen bonds between water molecules.



DRAW IT ► Draw partial charges on the water molecule at the far left, and draw three more water molecules hydrogen-bonded to it.

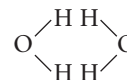


Animation: Polarity of Water

are hydrogen-bonded to their neighbors. The extraordinary properties of water emerge from this hydrogen bonding, which organizes water molecules into a higher level of structural order.

CONCEPT CHECK 3.1

1. **MAKE CONNECTIONS** ► What is electronegativity, and how does it affect interactions between water molecules? (Review Figure 2.11.)
2. **VISUAL SKILLS** ► Look at Figure 3.2 and explain why the central water molecule can hydrogen bond to four (rather than three or five) other water molecules.
3. Why is it unlikely that two neighboring water molecules would be arranged like this?



4. **WHAT IF?** ► What would be the effect on the properties of the water molecule if oxygen and hydrogen had equal electronegativity?

For suggested answers, see Appendix A.

CONCEPT 3.2

Four emergent properties of water contribute to Earth's suitability for life

We will examine four emergent properties of water that contribute to Earth's suitability as an environment for life: cohesive behavior, ability to moderate temperature, expansion upon freezing, and versatility as a solvent.

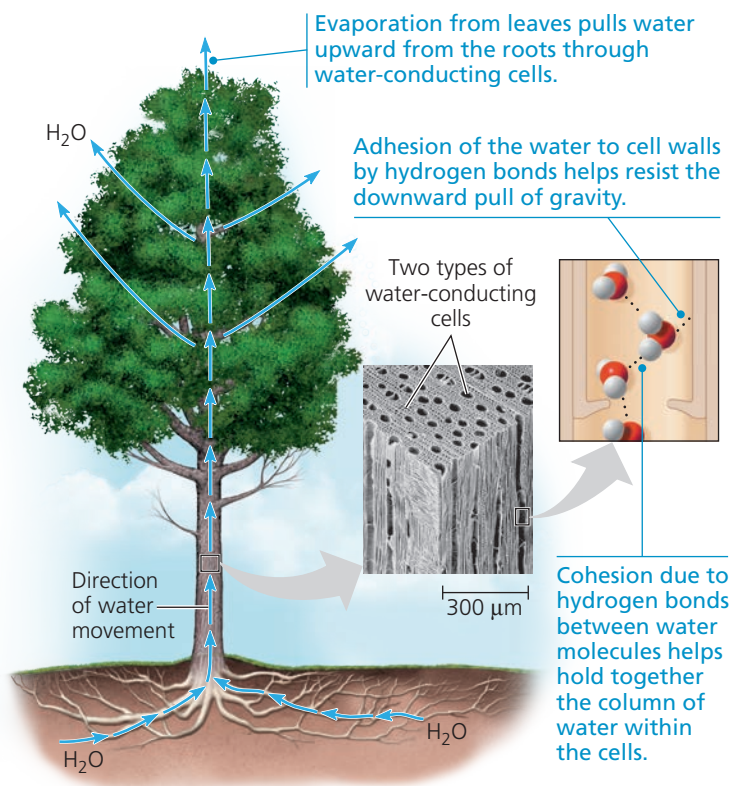
Cohesion of Water Molecules

Water molecules stay close to each other as a result of hydrogen bonding. Although the arrangement of molecules in a sample of liquid water is constantly changing, at any given moment many of the molecules are linked by multiple hydrogen bonds. These linkages make water more structured than most other liquids. Collectively, the hydrogen bonds hold the substance together, a phenomenon called **cohesion**.

Cohesion due to hydrogen bonding contributes to the transport of water and dissolved nutrients against gravity in plants. Water from the roots reaches the leaves through a network of water-conducting cells (Figure 3.3). As water evaporates from a leaf, hydrogen bonds cause water molecules leaving the veins to tug on molecules farther down, and the upward pull is transmitted through the water-conducting cells all the way to the roots. **Adhesion**, the clinging of one substance to another, also plays a role. Adhesion of water by hydrogen bonds to the molecules of cell walls helps counter the downward pull of gravity (see Figure 3.3).

Related to cohesion is **surface tension**, a measure of how difficult it is to stretch or break the surface of a liquid. At the interface between water and air is an ordered arrangement of water molecules, hydrogen-bonded to one another and to the water below, but not to the air above. This asymmetry gives

▼ **Figure 3.3 Water transport in plants.** Because of the properties of cohesion and adhesion, the tallest trees can transport water more than 100 m upward—approximately one-quarter the height of the Empire State Building in New York City.

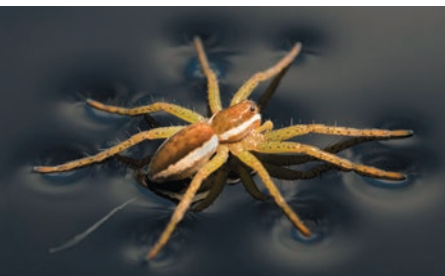


BioFlix® Animation: Adhesion and Cohesion in Plants
Animation: Cohesion of Water

water an unusually high surface tension, making it behave as though it were coated with an invisible film. You can observe the surface tension of water by slightly overfilling a drinking glass; the water will stand above the rim. The spider in **Figure 3.4** takes advantage of the surface tension of water to walk across a pond without breaking the surface.

Moderation of Temperature by Water

Water moderates air temperature by absorbing heat from air that is warmer and releasing the stored heat to air that is cooler. Water is effective as a heat bank because it can absorb or release a relatively large amount of heat with only a slight change in its own temperature. To understand this capability of water, let's first look at temperature and heat.



◀ **Figure 3.4 Walking on water.** The high surface tension of water, resulting from the collective strength of its hydrogen bonds, allows this raft spider to walk on the surface of a pond.

Temperature and Heat

Anything that moves has **kinetic energy**, the energy of motion. Atoms and molecules have kinetic energy because they are always moving, although not necessarily in any particular direction. The faster a molecule moves, the greater its kinetic energy. The kinetic energy associated with the random movement of atoms or molecules is called **thermal energy**. Thermal energy is related to temperature, but they are not the same thing. **Temperature** represents the *average* kinetic energy of the molecules in a body of matter, regardless of volume, whereas the thermal energy of a body of matter reflects the *total* kinetic energy, and thus depends on the matter's volume. When water is heated in a coffeemaker, the average speed of the molecules increases, and the thermometer records this as a rise in temperature of the liquid. The total amount of thermal energy also increases in this case. Note, however, that although the pot of coffee has a much higher temperature than, say, the water in a swimming pool, the swimming pool contains more thermal energy because of its much greater volume.

Whenever two objects of different temperature are brought together, thermal energy passes from the warmer to the cooler object until the two are the same temperature. Molecules in the cooler object speed up at the expense of the thermal energy of the warmer object. An ice cube cools a drink not by adding coldness to the liquid, but by absorbing thermal energy from the liquid as the ice itself melts. Thermal energy in transfer from one body of matter to another is defined as **heat**.

One convenient unit of heat used in this book is the **calorie (cal)**. A calorie is the amount of heat it takes to raise the temperature of 1 g of water by 1°C. Conversely, a calorie is also the amount of heat that 1 g of water releases when it cools by 1°C. A **kilocalorie (kcal)**, 1,000 cal, is the quantity of heat required to raise the temperature of 1 kilogram (kg) of water by 1°C. (The "Calories" on food packages are actually kilocalories.) Another energy unit used in this book is the **joule (J)**. One joule equals 0.239 cal; one calorie equals 4.184 J.

Water's High Specific Heat

The ability of water to stabilize temperature stems from its relatively high specific heat. The **specific heat** of a substance is defined as the amount of heat that must be absorbed or lost for 1 g of that substance to change its temperature by 1°C. We already know water's specific heat because we have defined a calorie as the amount of heat that causes 1 g of water to change its temperature by 1°C. Therefore, the specific heat of water is 1 calorie per gram and per degree Celsius, abbreviated as 1 cal/(g · °C). Compared with most other substances, water has an unusually high specific heat. For example, ethyl alcohol, the type of alcohol in alcoholic beverages, has a specific heat of 0.6 cal/(g · °C); that is, only 0.6 cal is required to raise the temperature of 1 g of ethyl alcohol by 1°C.

Because of the high specific heat of water relative to other materials, water will change its temperature less than other

liquids when it absorbs or loses a given amount of heat. The reason you can burn your fingers by touching the side of an iron pot on the stove when the water in the pot is still luke-warm is that the specific heat of water is ten times greater than that of iron. In other words, the same amount of heat will raise the temperature of 1 g of the iron much faster than it will raise the temperature of 1 g of the water. Specific heat can be thought of as a measure of how well a substance resists changing its temperature when it absorbs or releases heat. Water resists changing its temperature; when it does change its temperature, it absorbs or loses a relatively large quantity of heat for each degree of change.

We can trace water's high specific heat, like many of its other properties, to hydrogen bonding. Heat must be absorbed in order to break hydrogen bonds; by the same token, heat is released when hydrogen bonds form. A calorie of heat causes a relatively small change in the temperature of water because much of the heat is used to disrupt hydrogen bonds before the water molecules can begin moving faster. And when the temperature of water drops slightly, many additional hydrogen bonds form, releasing a considerable amount of energy in the form of heat.

What is the relevance of water's high specific heat to life on Earth? A large body of water can absorb and store a huge amount of heat from the sun in the daytime and during summer while warming up only a few degrees. At night and during winter, the gradually cooling water can warm the air. This capability of water serves to moderate air temperatures in coastal areas (**Figure 3.5**). The high specific heat of water also tends to stabilize ocean temperatures, creating a favorable environment for marine life. Thus, because of its high specific heat, the water that covers most of Earth keeps temperature fluctuations on land and in water within limits that permit life. Also, because organisms are made primarily of water, they are better able to resist changes in their own temperature than if they were made of a liquid with a lower specific heat.

Evaporative Cooling

Molecules of any liquid stay close together because they are attracted to one another. Molecules moving fast enough to

overcome these attractions can depart the liquid and enter the air as a gas (vapor). This transformation from a liquid to a gas is called vaporization, or evaporation. Recall that the speed of molecular movement varies and that temperature is the *average* kinetic energy of molecules. Even at low temperatures, the speediest molecules can escape into the air. Some evaporation occurs at any temperature; a glass of water at room temperature, for example, will eventually evaporate completely. If a liquid is heated, the average kinetic energy of molecules increases and the liquid evaporates more rapidly.

Heat of vaporization is the quantity of heat a liquid must absorb for 1 g of it to be converted from the liquid to the gaseous state. For the same reason that water has a high specific heat, it also has a high heat of vaporization relative to most other liquids. To evaporate 1 g of water at 25°C, about 580 cal of heat is needed—nearly double the amount needed to vaporize a gram of alcohol or ammonia. Water's high heat of vaporization is another emergent property resulting from the strength of its hydrogen bonds, which must be broken before the molecules can exit from the liquid in the form of water vapor.

The high amount of energy required to vaporize water has a wide range of effects. On a global scale, for example, it helps moderate Earth's climate. A considerable amount of solar heat absorbed by tropical seas is consumed during the evaporation of surface water. Then, as moist tropical air circulates poleward, it releases heat as it condenses and forms rain. On an organismal level, water's high heat of vaporization accounts for the severity of steam burns. These burns are caused by the heat energy released when steam condenses into liquid on the skin.

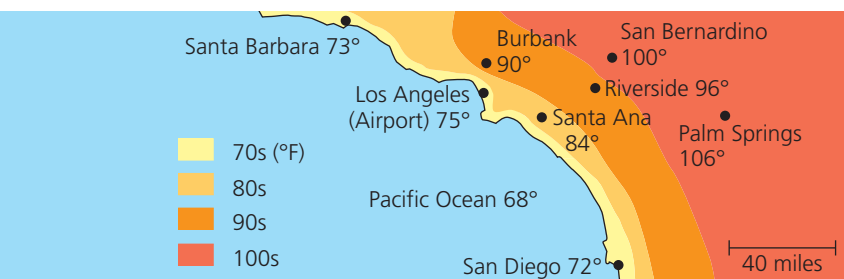
As a liquid evaporates, the surface of the liquid that remains behind cools down (its temperature decreases). This **evaporative cooling** occurs because the "hottest" molecules, those with the greatest kinetic energy, are the most likely to leave as gas. It is as if the 100 fastest runners at a college transferred to another school; the average speed of the remaining students would decline.

Evaporative cooling of water contributes to the stability of temperature in lakes and ponds and also provides a mechanism that prevents terrestrial organisms from overheating. For example, evaporation of water from the leaves of a plant helps keep the tissues in the leaves from becoming too warm in the sunlight. Evaporation of sweat from human skin dissipates body heat and helps prevent overheating on a hot day or when excess heat is generated by strenuous activity. High humidity on a hot day increases discomfort because the high concentration of water vapor in the air inhibits the evaporation of sweat from the body.

Floating of Ice on Liquid Water

Water is one of the few substances that are less dense as a solid than as a liquid. In other words, ice floats on liquid water. While other materials contract and become denser when they solidify, water expands. The cause of this exotic behavior is, once again,

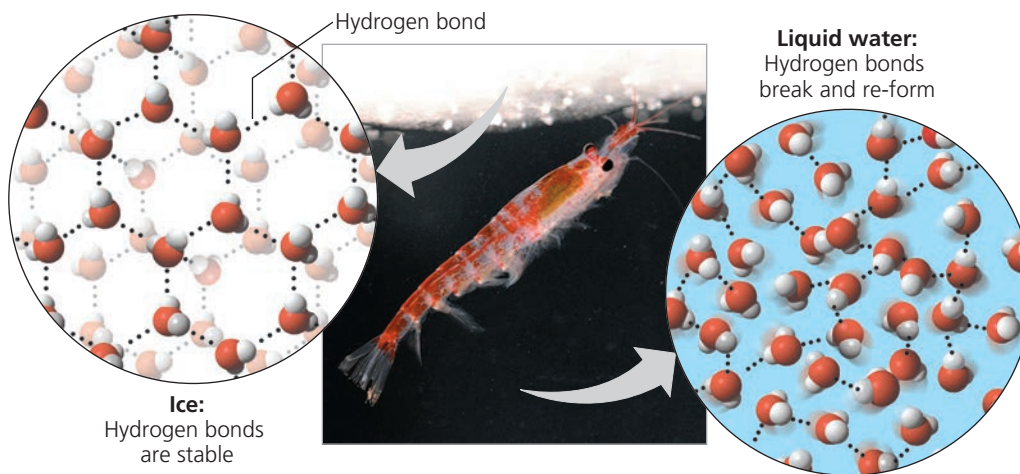
▼ **Figure 3.5** Temperatures for the Pacific Ocean and Southern California on an August day.



INTERPRET THE DATA ► Explain the pattern of temperatures shown in this diagram.

► **Figure 3.6 Ice: crystalline structure and floating barrier.** In ice, each molecule is hydrogen-bonded to four neighbors in a three-dimensional crystal. Because the crystal is spacious, ice has fewer molecules than an equal volume of liquid water. In other words, ice is less dense than liquid water. Floating ice becomes a barrier that insulates the liquid water below from the colder air. The marine organism shown here is a type of shrimp called krill; it was photographed beneath floating ice in the Southern Ocean near Antarctica.

WHAT IF? ► If water did not form hydrogen bonds, what would happen to the shrimp's habitat, shown here?

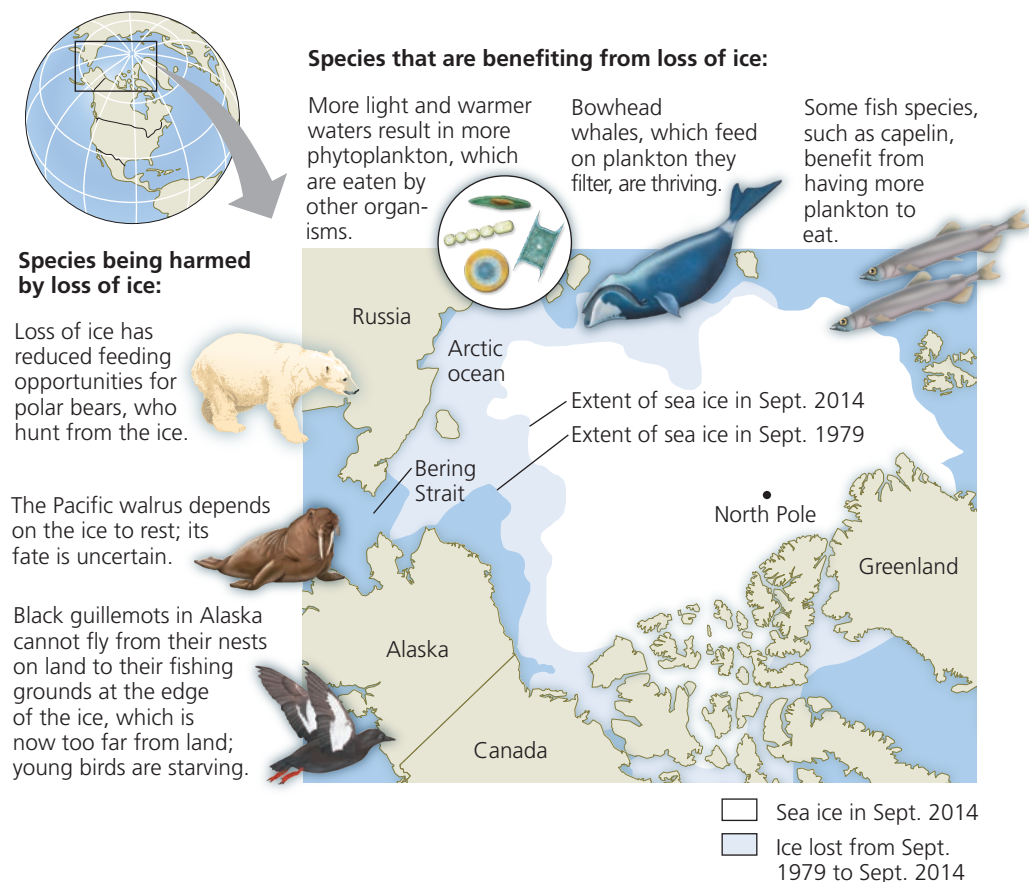


hydrogen bonding. At temperatures above 4°C, water behaves like other liquids, expanding as it warms and contracting as it cools. As the temperature falls from 4°C to 0°C, water begins to freeze because more and more of its molecules are moving too slowly to break hydrogen bonds. At 0°C, the molecules become locked into a crystalline lattice, each water molecule hydrogen-bonded to four partners (**Figure 3.6**). The hydrogen bonds keep the molecules at “arm’s length,” far enough apart to make ice about 10% less dense (10% fewer molecules in the same volume) than liquid water at 4°C. When ice absorbs enough heat for its temperature to rise above 0°C, hydrogen bonds between molecules are disrupted. As the crystal collapses, the ice melts and molecules have fewer hydrogen bonds, allowing them to slip closer together. Water reaches its greatest density at 4°C and then begins to expand as the molecules move faster. Even in liquid water, many of the molecules are connected by hydrogen bonds, though only transiently: The hydrogen bonds are constantly breaking and re-forming.

The ability of ice to float due to its lower density is an important factor in the suitability of the environment for life. If ice sank, then eventually all ponds, lakes, and even oceans would freeze solid, making life as we know it impossible on Earth. During summer, only the upper few inches of the ocean would thaw. Instead, when a deep body of water cools, the floating ice insulates the liquid water below, preventing it from freezing and allowing life to exist under the frozen surface, as shown in the photo in **Figure 3.6**. Besides insulating the water below, ice also provides a solid habitat for some animals, such as polar bears and seals.

Many scientists are worried that these bodies of ice are at risk of disappearing. Global warming, which is caused by carbon dioxide and other “greenhouse” gases in the atmosphere (see **Figure 56.28**), is having a profound effect on icy environments around the globe. In the Arctic, the average air temperature has risen 2.2°C just since 1961. This temperature increase has affected the seasonal balance between Arctic sea ice and liquid water, causing ice to form later in the year, to melt earlier, and to cover a smaller area. The rate at which glaciers and Arctic sea ice are disappearing is posing an extreme challenge to animals that depend on ice for their survival (**Figure 3.7**).

▼ **Figure 3.7 Effects of climate change on the Arctic.** Warmer temperatures in the Arctic cause more sea ice to melt in the summer, benefiting some organisms and harming others.



Water: The Solvent of Life

A sugar cube placed in a glass of water will dissolve with a little stirring. The glass will then contain a uniform mixture of sugar and water; the concentration of dissolved sugar will be the same everywhere in the mixture. A liquid that is a completely homogeneous mixture of two or more substances is called a **solution**. The dissolving agent of a solution is the **solvent**, and the substance that is dissolved is the **solute**. In this case, water is the solvent and sugar is the solute. An **aqueous solution** is one in which the solute is dissolved in water; water is the solvent.

Water is a very versatile solvent, a quality we can trace to the polarity of the water molecule. Suppose, for example, that a spoonful of table salt, the ionic compound sodium chloride (NaCl), is placed in water (**Figure 3.8**). At the surface of each crystal of salt, the sodium and chloride ions are exposed to the solvent. These ions and regions of the water molecules are attracted to each other due to their opposite charges. The oxygens of the water molecules have regions of partial negative charge that are attracted to sodium cations. The hydrogen regions are partially positively charged and are attracted to chloride anions. As a result, water molecules surround the individual sodium and chloride ions, separating and shielding them from one another. The sphere of water molecules around each dissolved ion is called a **hydration shell**. Working inward from the surface of each salt crystal, water eventually dissolves all the ions. The result is a solution of two solutes, sodium cations and chloride anions, homogeneously mixed with water, the solvent. Other ionic compounds also dissolve in water. Seawater, for instance, contains a great variety of dissolved ions, as do living cells.

A compound does not need to be ionic to dissolve in water; many compounds made up of nonionic polar molecules,

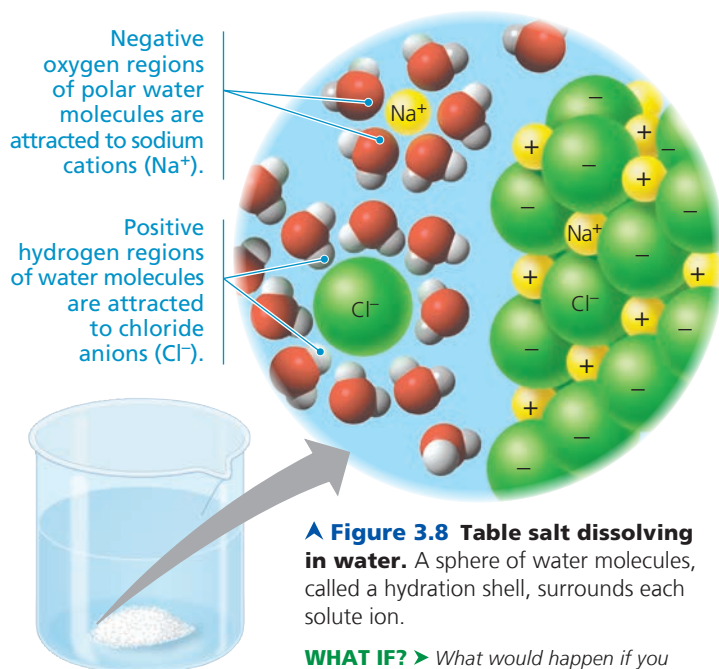
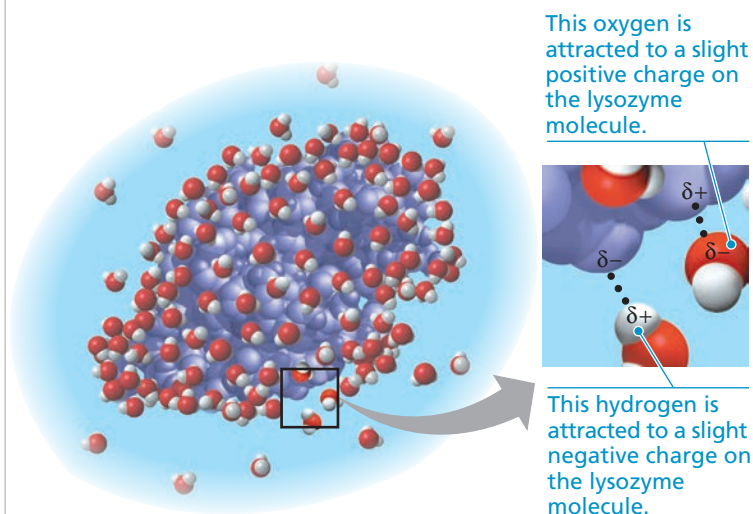


Figure 3.9 A water-soluble protein. Human lysozyme is a protein found in tears and saliva that has antibacterial action (see Figure 5.16). This model shows the lysozyme molecule (purple) in an aqueous environment. Ionic and polar regions on the protein's surface attract the partially charged regions on water molecules.



such as the sugar in the sugar cube mentioned earlier, are also water-soluble. Such compounds dissolve when water molecules surround each of the solute molecules, forming hydrogen bonds with them. Even molecules as large as proteins can dissolve in water if they have ionic and polar regions on their surface (**Figure 3.9**). Many different kinds of polar compounds are dissolved (along with ions) in the water of such biological fluids as blood, the sap of plants, and the liquid within all cells. Water is the solvent of life.

Hydrophilic and Hydrophobic Substances

Any substance that has an affinity for water is said to be **hydrophilic** (from the Greek *hydro*, water, and *philos*, loving). In some cases, substances can be hydrophilic without actually dissolving. For example, some molecules in cells are so large that they do not dissolve. Another example of a hydrophilic substance that does not dissolve is cotton, a plant product. Cotton consists of giant molecules of cellulose, a compound with numerous regions of partial positive and partial negative charges that can form hydrogen bonds with water. Water adheres to the cellulose fibers. Thus, a cotton towel does a great job of drying the body, yet it does not dissolve in the washing machine. Cellulose is also present in the walls of water-conducting cells in a plant; you read earlier how the adhesion of water to these hydrophilic walls helps water move up the plant against gravity.

There are, of course, substances that do not have an affinity for water. Substances that are nonionic and nonpolar (or otherwise cannot form hydrogen bonds) actually seem to repel water; these substances are said to be **hydrophobic** (from the Greek *phobos*, fearing). An example from the kitchen is vegetable oil, which, as you know, does not mix stably with water-based substances such as vinegar. The hydrophobic

behavior of the oil molecules results from a prevalence of relatively nonpolar covalent bonds, in this case bonds between carbon and hydrogen, which share electrons almost equally. Hydrophobic molecules related to oils are major ingredients of cell membranes. (Imagine what would happen to a cell if its membrane dissolved!)

Solute Concentration in Aqueous Solutions

Most of the chemical reactions in organisms involve solutes dissolved in water. To understand such reactions, we must know how many atoms and molecules are involved and calculate the concentration of solutes in an aqueous solution (the number of solute molecules in a volume of solution).

When carrying out experiments, we use mass to calculate the number of molecules. We must first calculate the **molecular mass**, which is the sum of the masses of all the atoms in a molecule. As an example, let's calculate the molecular mass of table sugar (sucrose), $C_{12}H_{22}O_{11}$, by multiplying the number of atoms by the atomic mass of each element (see Appendix B). In round numbers of daltons, the mass of a carbon atom is 12, the mass of a hydrogen atom is 1, and the mass of an oxygen atom is 16. Thus, sucrose has a molecular mass of $(12 \times 12) + (22 \times 1) + (11 \times 16) = 342$ daltons. Because we can't weigh out small numbers of molecules, we usually measure substances in units called moles. Just as a dozen always means 12 objects, a **mole (mol)** represents an exact number of objects: 6.02×10^{23} , which is called Avogadro's number. Because of the way in which Avogadro's number and the unit *dalton* were originally defined, there are 6.02×10^{23} daltons in 1 g. Once we determine the molecular mass of a molecule such as sucrose, we can use the same number (342), but with the unit *gram*, to represent the mass of 6.02×10^{23} molecules of sucrose, or 1 mol of sucrose (this is sometimes called the *molar mass*). To obtain 1 mol of sucrose in the lab, therefore, we weigh out 342 g.

The practical advantage of measuring a quantity of chemicals in moles is that a mole of one substance has exactly the same number of molecules as a mole of any other substance. If the molecular mass of substance A is 342 daltons and that of substance B is 10 daltons, then 342 g of A will have the same number of molecules as 10 g of B. A mole of ethyl alcohol (C_2H_6O) also contains 6.02×10^{23} molecules, but its mass is only 46 g because the mass of a molecule of ethyl alcohol is less than that of a molecule of sucrose. Measuring in moles makes it convenient for scientists working in the laboratory to combine substances in fixed ratios of molecules.

How would we make a liter (L) of solution consisting of 1 mol of sucrose dissolved in water? We would measure out 342 g of sucrose and then gradually add water, while stirring, until the sugar was completely dissolved. We would then add enough water to bring the total volume of the solution up to 1 L. At that point, we would have a 1-molar (1 M) solution of sucrose. **Molarity**—the number of moles of solute per liter of solution—is the unit of concentration most often used by biologists for aqueous solutions.

Water's capacity as a versatile solvent complements the other properties discussed in this chapter. Since these remarkable properties allow water to support life on Earth so well, scientists who seek life elsewhere in the universe look for water as a sign that a planet might sustain life.

MP3 Tutor: The Properties of Water

Possible Evolution of Life on Other Planets

EVOLUTION Biologists who look for life elsewhere in the universe (known as *astrobiologists*) have concentrated their search on planets that might have water. More than 800 planets have been found outside our solar system, and there is evidence for the presence of water vapor on a few of them. In our own solar system, Mars has been a focus of study. Like Earth, Mars has an ice cap at both poles. Images from spacecraft sent to Mars showed that ice is present just under the surface of Mars and enough water vapor exists in its atmosphere for frost to form. In 2015, scientists found evidence of water flowing on Mars (**Figure 3.10**), and other studies suggested conditions existed that could have supported microorganismal life. Drilling below the surface may be the next step in the search for signs of life on Mars. If any life-forms or fossils are found, their study will shed light on the process of evolution from an entirely new perspective.

▼ Figure 3.10 Evidence for liquid water on Mars. Water appears to have helped form these dark streaks that run downhill on Mars during the summer. NASA scientists also found evidence of hydrated salts, indicating water is present. (This digitally treated photograph was taken by the Mars Reconnaissance Orbiter.)



CONCEPT CHECK 3.2

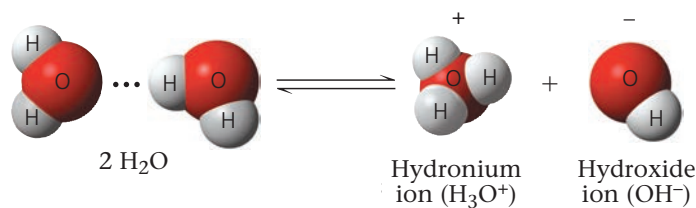
1. Describe how properties of water contribute to the upward movement of water in a tree.
2. Explain the saying "It's not the heat; it's the humidity."
3. How can the freezing of water crack boulders?
4. **WHAT IF? >** Dishwashing soaps are made up of molecules with one hydrophilic end and one hydrophobic end. How does this help to clean greasy utensils? What would happen if soaps lose their hydrophobic property?
5. **INTERPRET THE DATA >** The concentration of the appetite-regulating hormone ghrelin is about 1.3×10^{-10} M in the blood of a fasting person. How many molecules of ghrelin are in 1 L of blood?

For selected answers, see Appendix A.

CONCEPT 3.3

Acidic and basic conditions affect living organisms

Occasionally, a hydrogen atom participating in a hydrogen bond between two water molecules shifts from one molecule to the other. When this happens, the hydrogen atom leaves its electron behind, and what is actually transferred is a **hydrogen ion** (H^+), a single proton with a charge of 1+. The water molecule that lost a proton is now a **hydroxide ion** (OH^-), which has a charge of 1-. The proton binds to the other water molecule, making that molecule a **hydronium ion** (H_3O^+). We can picture the chemical reaction as follows:



Animation: Dissociation of Water Molecules

By convention, H^+ (the hydrogen ion) is used to represent H_3O^+ (the hydronium ion), and we follow that practice in this book. Keep in mind, though, that H^+ does not exist on its own in an aqueous solution. It is always associated with a water molecule in the form of H_3O^+ .

As indicated by the double arrows, this is a reversible reaction that reaches a state of dynamic equilibrium when water molecules dissociate at the same rate that they are being reformed from H^+ and OH^- . At this equilibrium point, the concentration of water molecules greatly exceeds the concentrations of H^+ and OH^- . In pure water, only one water molecule in every 554 million is dissociated; the concentration of H^+ and of OH^- in pure water is therefore 10^{-7} M (at 25°C). This means there is only one ten-millionth of a mole of hydrogen ions per liter of pure water and an equal number of hydroxide ions. (Even so, this is a huge number—over 60,000 *trillion*—of each ion in a liter of pure water.)

Although the dissociation of water is reversible and statistically rare, it is exceedingly important in the chemistry of life. H^+ and OH^- are very reactive. Changes in their concentrations can drastically affect a cell's proteins and other complex molecules. As we have seen, the concentrations of H^+ and OH^- are equal in pure water, but adding certain kinds of solutes, called acids and bases, disrupts this balance. Biologists use something called the pH scale to describe how acidic or basic (the opposite of acidic) a solution is. In the remainder of this chapter, you will learn about acids, bases, and pH and why changes in pH can adversely affect organisms.

Acids and Bases

What would cause an aqueous solution to have an imbalance in H^+ and OH^- concentrations? When acids dissolve in water, they donate additional H^+ to the solution. An **acid** is a substance that increases the hydrogen ion concentration of a solution. For example, when hydrochloric acid (HCl) is added to water, hydrogen ions dissociate from chloride ions:



This source of H^+ (dissociation of water is the other source) results in an acidic solution—one having more H^+ than OH^- .

A substance that reduces the hydrogen ion concentration of a solution is called a **base**. Some bases reduce the H^+ concentration directly by accepting hydrogen ions. Ammonia (NH_3), for instance, acts as a base when the unshared electron pair in nitrogen's valence shell attracts a hydrogen ion from the solution, resulting in an ammonium ion (NH_4^+):



Other bases reduce the H^+ concentration indirectly by dissociating to form hydroxide ions, which combine with hydrogen ions and form water. One such base is sodium hydroxide (NaOH), which in water dissociates into its ions:



In either case, the base reduces the H^+ concentration. Solutions with a higher concentration of OH^- than H^+ are known as basic solutions. A solution in which the H^+ and OH^- concentrations are equal is said to be neutral.

Notice that single arrows were used in the reactions for HCl and NaOH . These compounds dissociate completely when mixed with water, so hydrochloric acid is called a strong acid and sodium hydroxide a strong base. In contrast, ammonia is a weak base. The double arrows in the reaction for ammonia indicate that the binding and release of hydrogen ions are reversible reactions, although at equilibrium there will be a fixed ratio of NH_4^+ to NH_3 .

Weak acids are acids that reversibly release and accept back hydrogen ions. An example is carbonic acid:



Here the equilibrium so favors the reaction in the left direction that when carbonic acid is added to pure water, only 1% of the molecules are dissociated at any particular time. Still, that is enough to shift the balance of H^+ and OH^- from neutrality.

The pH Scale

In any aqueous solution at 25°C , the product of the H^+ and OH^- concentrations is constant at 10^{-14} . This can be written

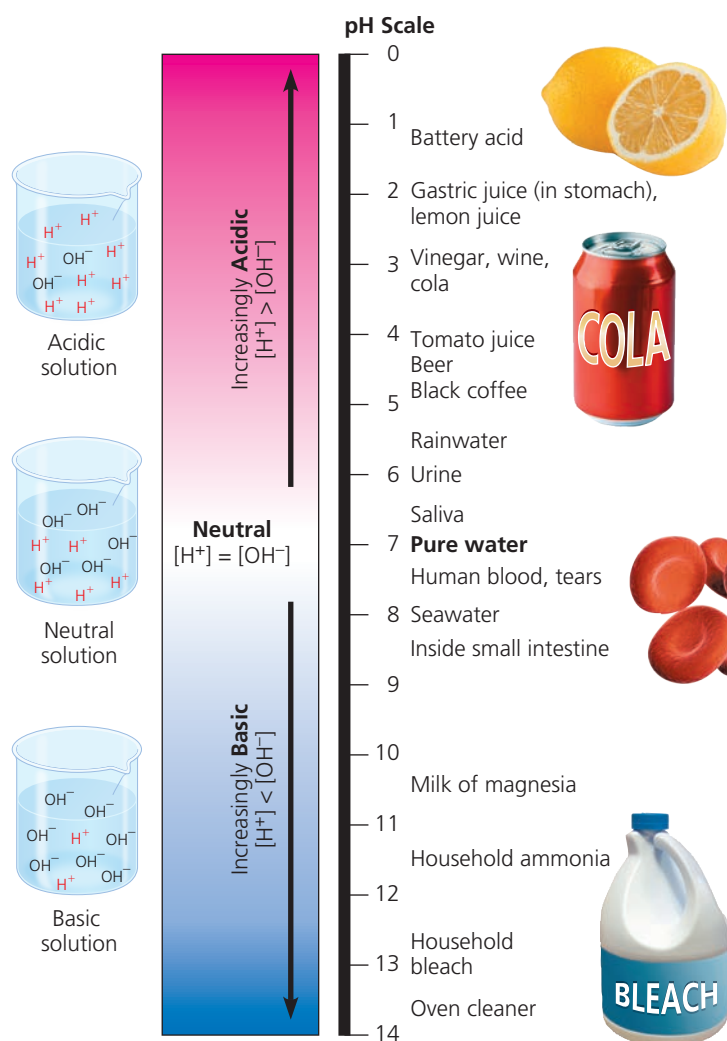
$$[\text{H}^+][\text{OH}^-] = 10^{-14}$$

(The brackets indicate molar concentration.) As previously mentioned, in a neutral solution at 25°C , $[\text{H}^+] = 10^{-7}$ and $[\text{OH}^-] = 10^{-7}$. Therefore, the product of $[\text{H}^+]$ and $[\text{OH}^-]$ in a

neutral solution at 25°C is 10^{-14} . If enough acid is added to a solution to increase $[H^+]$ to $10^{-5} M$, then $[OH^-]$ will decline by an equivalent factor to $10^{-9} M$ (note that $10^{-5} \times 10^{-9} = 10^{-14}$). This constant relationship expresses the behavior of acids and bases in an aqueous solution. An acid not only adds hydrogen ions to a solution, but also removes hydroxide ions because of the tendency for H^+ to combine with OH^- , forming water. A base has the opposite effect, increasing OH^- concentration but also reducing H^+ concentration by the formation of water. If enough of a base is added to raise the OH^- concentration to $10^{-4} M$, it will cause the H^+ concentration to drop to $10^{-10} M$. Whenever we know the concentration of either H^+ or OH^- in an aqueous solution, we can deduce the concentration of the other ion.

Because the H^+ and OH^- concentrations of solutions can vary by a factor of 100 trillion or more, scientists have developed a way to express this variation more conveniently than in moles per liter. The pH scale (Figure 3.11) compresses the range of H^+ and OH^- concentrations by employing logarithms.

▼ **Figure 3.11** The pH scale and pH values of some aqueous solutions.



The **pH** of a solution is defined as the negative logarithm (base 10) of the hydrogen ion concentration:

$$\text{pH} = -\log [H^+]$$

For a neutral aqueous solution, $[H^+]$ is $10^{-7} M$, giving us

$$-\log 10^{-7} = -(-7) = 7$$

Notice that pH *decreases* as H^+ concentration *increases* (see Figure 3.11). Notice, too, that although the pH scale is based on H^+ concentration, it also implies OH^- concentration. A solution of pH 10 has a hydrogen ion concentration of $10^{-10} M$ and a hydroxide ion concentration of $10^{-4} M$.

The pH of a neutral aqueous solution at 25°C is 7, the midpoint of the pH scale. A pH value less than 7 denotes an acidic solution; the lower the number, the more acidic the solution. The pH for basic solutions is above 7. Most biological fluids, such as blood and saliva, are within the range of pH 6–8. There are a few exceptions, however, including the strongly acidic digestive juice of the human stomach (gastric juice), which has a pH of about 2.

Remember that each pH unit represents a tenfold difference in H^+ and OH^- concentrations. It is this mathematical feature that makes the pH scale so compact. A solution of pH 3 is not twice as acidic as a solution of pH 6, but 1,000 times ($10 \times 10 \times 10$) more acidic. When the pH of a solution changes slightly, the actual concentrations of H^+ and OH^- in the solution change substantially.

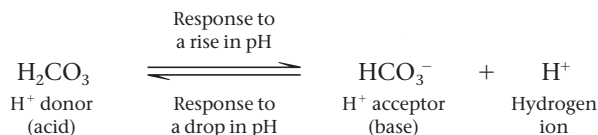
Buffers

The internal pH of most living cells is close to 7. Even a slight change in pH can be harmful because the chemical processes of the cell are very sensitive to the concentrations of hydrogen and hydroxide ions. The pH of human blood is very close to 7.4, which is slightly basic. A person cannot survive for more than a few minutes if the blood pH drops to 7 or rises to 7.8, and a chemical system exists in the blood that maintains a stable pH. If 0.01 mol of a strong acid is added to a liter of pure water, the pH drops from 7.0 to 2.0. If the same amount of acid is added to a liter of blood, however, the pH decrease is only from 7.4 to 7.3. Why does the addition of acid have so much less of an effect on the pH of blood than it does on the pH of water?

The presence of substances called buffers allows biological fluids to maintain a relatively constant pH despite the addition of acids or bases. A **buffer** is a substance that minimizes changes in the concentrations of H^+ and OH^- in a solution. It does so by accepting hydrogen ions from the solution when they are in excess and donating hydrogen ions to the solution when they have been depleted. Most buffer solutions contain a weak acid and its corresponding base, which combine reversibly with hydrogen ions.

Several buffers contribute to pH stability in human blood and many other biological solutions. One of these is carbonic

acid (H_2CO_3), which is formed when CO_2 reacts with water in blood plasma. As mentioned earlier, carbonic acid dissociates to yield a bicarbonate ion (HCO_3^-) and a hydrogen ion (H^+):



The chemical equilibrium between carbonic acid and bicarbonate acts as a pH regulator, the reaction shifting left or right as other processes in the solution add or remove hydrogen ions. If the H^+ concentration in blood begins to fall (that is, if pH rises), the reaction proceeds to the right and more carbonic acid dissociates, replenishing hydrogen ions. But when the H^+ concentration in blood begins to rise (when pH drops), the reaction proceeds to the left, with HCO_3^- (the base) removing the hydrogen ions from the solution and forming H_2CO_3 . Thus, the carbonic acid–bicarbonate buffering system consists of an acid and a base in equilibrium with each other. Most other buffers are also acid–base pairs.

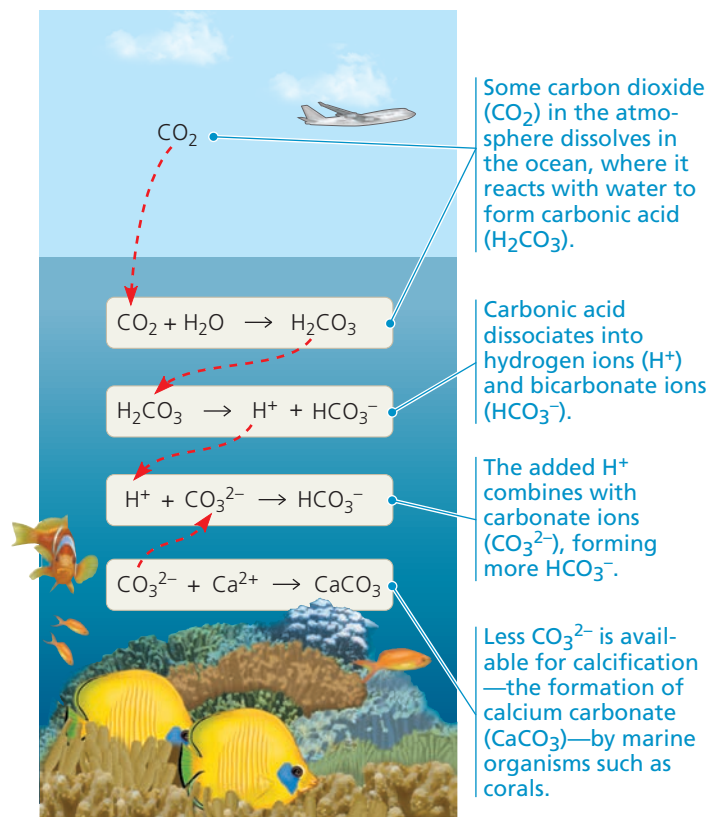
Acidification: A Threat to Our Oceans

Among the many threats to water quality posed by human activities is the burning of fossil fuels, which releases CO_2 into the atmosphere. The resulting increase in atmospheric CO_2 levels has caused global warming and other aspects of climate change (see Concept 56.4). In addition, about 25% of human-generated CO_2 is absorbed by the oceans. In spite of the huge volume of water in the oceans, scientists worry that the absorption of so much CO_2 will harm marine ecosystems.

Recent data have shown that such fears are well founded. When CO_2 dissolves in seawater, it reacts with water to form carbonic acid, which lowers ocean pH. This process, known as **ocean acidification**, alters the delicate balance of conditions for life in the oceans (Figure 3.12). Based on measurements of CO_2 levels in air bubbles trapped in ice over thousands of years, scientists calculate that the pH of the oceans is 0.1 pH unit lower now than at any time in the past 420,000 years. Recent studies predict that it will drop another 0.3–0.5 pH unit by the end of this century.

As seawater acidifies, the extra hydrogen ions combine with carbonate ions (CO_3^{2-}) to form bicarbonate ions (HCO_3^-), thereby reducing the carbonate ion concentration (see Figure 3.12). Scientists predict that ocean acidification will cause the carbonate ion concentration to decrease by 40% by the year 2100. This is of great concern because carbonate ions are required for calcification, the production of calcium carbonate (CaCO_3) by many marine organisms, including reef-building corals and animals that build shells. The **Scientific Skills Exercise** allows you to work with data from an experiment examining the effect of carbonate ion concentration on coral reefs. Coral reefs are sensitive ecosystems that act as havens for a great diversity of marine life.

▼ **Figure 3.12** Atmospheric CO_2 from human activities and its fate in the ocean.



VISUAL SKILLS ► Looking at all the chemical equations above, summarize the effect of adding excess CO_2 to the oceans on the calcification process in the final equation.



ABC News Video:
Ocean Acidification

The disappearance of coral reef ecosystems would be a tragic loss of biological diversity.

If there is any reason for optimism about the future quality of water resources on our planet, it is that we have made progress in learning about the delicate chemical balances in oceans, lakes, and rivers. Continued progress can come only from the actions of informed individuals, like yourselves, who are concerned about environmental quality. This requires understanding the crucial role that water plays in the suitability of the environment for continued life on Earth.

CONCEPT CHECK 3.3

1. Compared with a basic solution at pH 9, the same volume of an acidic solution at pH 4 has _____ times as many hydrogen ions (H^+).
2. Is the pH of 0.01 M HCl the same as that of 0.001 M HCl? Explain.
3. Acetic acid (CH_3COOH) can be a buffer, similar to carbonic acid. Write the dissociation reaction, identifying the acid, base, H^+ acceptor, and H^+ donor.
4. **WHAT IF?** ► What would happen to a solution of acetic acid if a strong base like NaOH is added to it? Use the reaction from question 3 to explain the result.

For suggested answers, see Appendix A.

SCIENTIFIC SKILLS EXERCISE

Interpreting a Scatter Plot with a Regression Line

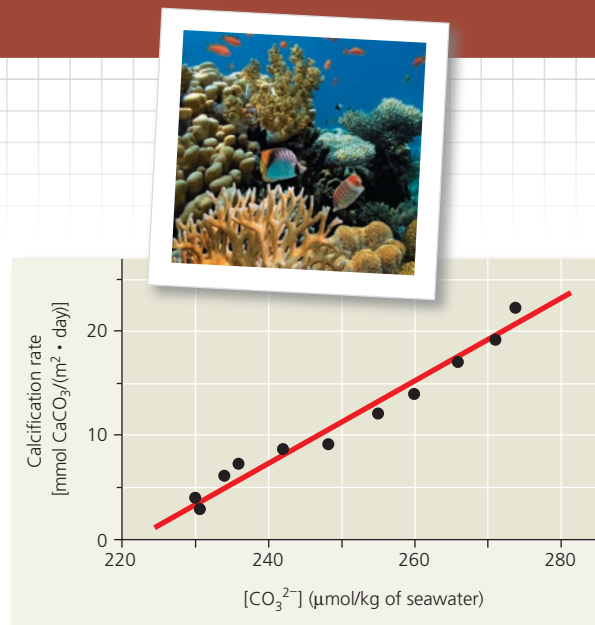
How Does the Carbonate Ion Concentration of Seawater Affect the Calcification Rate of a Coral Reef? Scientists predict that acidification of the ocean due to higher levels of atmospheric CO_2 will lower the concentration of dissolved carbonate ions, which living corals use to build calcium carbonate reef structures. In this exercise, you will analyze data from a controlled experiment that examined the effect of carbonate ion concentration ($[\text{CO}_3^{2-}]$) on calcium carbonate deposition, a process called calcification.

How the Experiment Was Done For several years, scientists conducted research on ocean acidification using a large coral reef aquarium at Biosphere 2 in Arizona. They measured the rate of calcification by the reef organisms and examined how the calcification rate changed with differing amounts of dissolved carbonate ions in the seawater.

Data from the Experiment The black data points in the graph form a scatter plot. The red line, known as a linear regression line, is the best-fitting straight line for these points.

INTERPRET THE DATA

- When presented with a graph of experimental data, the first step in analysis is to determine what each axis represents. (a) In words, explain what is being shown on the x-axis. Be sure to include the units. (b) What is being shown on the y-axis (including units)? (c) Which variable is the independent variable—the variable that was *manipulated* by the researchers? (d) Which variable is the dependent variable—the variable that responded to or depended on the treatment, which was *measured* by the researchers? (For additional information about graphs, see the Scientific Skills Review in Appendix F.)
- Based on the data shown in the graph, describe in words the relationship between carbonate ion concentration and calcification rate.
- (a) If the seawater carbonate ion concentration is $270 \mu\text{mol/kg}$, what is the approximate rate of calcification, and approximately how many days would it take 1 square meter of reef to accumulate 30 mmol of calcium carbonate (CaCO_3)? (b) If the seawater



Data from C. Langdon et al., Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef, *Global Biogeochemical Cycles* 14:639–654 (2000).

carbonate ion concentration is $250 \mu\text{mol/kg}$, what is the approximate rate of calcification, and approximately how many days would it take 1 square meter of reef to accumulate 30 mmol of calcium carbonate? (c) If carbonate ion concentration decreases, how does the calcification rate change, and how does that affect the time it takes coral to grow?

- (a) Referring to the equations in Figure 3.12, determine which step of the process is measured in this experiment. (b) Are the results of this experiment consistent with the hypothesis that increased atmospheric $[\text{CO}_2]$ will slow the growth of coral reefs? Why or why not?



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

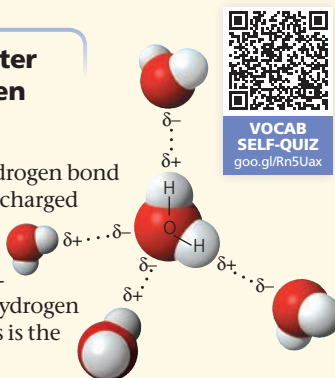
3 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 3.1

Polar covalent bonds in water molecules result in hydrogen bonding (p. 93)

- Water is a **polar molecule**. A hydrogen bond forms when a partially negatively charged region on the oxygen of one water molecule is attracted to the partially positively charged hydrogen of a nearby water molecule. Hydrogen bonding between water molecules is the basis for water's properties.



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

DRAW IT ➤ Label a hydrogen bond and a polar covalent bond in the diagram of five water molecules. Is a hydrogen bond a covalent bond? Explain.

CONCEPT 3.2

Four emergent properties of water contribute to Earth's suitability for life (pp. 93–98)

- Hydrogen bonding keeps water molecules close to each other, giving water **cohesion**. Hydrogen bonding is also responsible for water's **surface tension**.
- Water has a high **specific heat**: Heat is absorbed when hydrogen bonds break and is released when hydrogen bonds form. This helps keep **temperatures** relatively steady, within limits that permit life. **Evaporative cooling** is based on water's high **heat of vaporization**. The evaporative loss of the most energetic water molecules cools a surface.

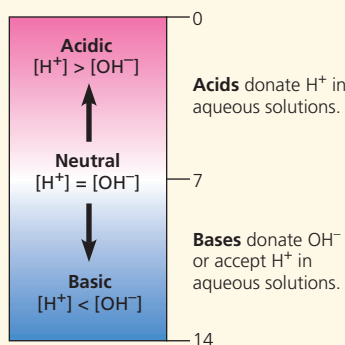
- Ice floats because it is less dense than liquid water. This property allows life to exist under the frozen surfaces of lakes and polar seas.
- Water is an unusually versatile **solvent** because its polar molecules are attracted to ions and polar substances that can form hydrogen bonds. **Hydrophilic** substances have an affinity for water; **hydrophobic** substances do not. **Molarity**, the number of moles of **solute** per liter of **solution**, is used as a measure of solute concentration in solutions. A **mole** is a certain number of molecules of a substance. The mass of a mole of a substance in grams is the same as the **molecular mass** in daltons.
- The emergent properties of water support life on Earth and may contribute to the potential for life to have evolved on other planets.

? Describe how different types of solutes dissolve in water. Explain what a solution is.

CONCEPT 3.3

Acidic and basic conditions affect living organisms (pp. 99–102)

- A water molecule can transfer an H^+ to another water molecule to form H_3O^+ (represented simply by H^+) and OH^- .
- The concentration of H^+ is expressed as **pH**; $pH = -\log [H^+]$. A **buffer** consists of an acid-base pair that combines reversibly with hydrogen ions, allowing it to resist pH changes.
- The burning of fossil fuels increases the amount of CO_2 in the atmosphere. Some CO_2 dissolves in the oceans, causing **ocean acidification**, which has potentially grave consequences for marine organisms that rely on calcification.



? Explain what happens to the concentration of hydrogen ions in an aqueous solution when you add a base and cause the concentration of OH^- to rise to 10^{-3} . What is the pH of this solution?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

- DRAW IT** Draw the hydration shells that form around a potassium ion and a chloride ion when potassium chloride (KCl) dissolves in water. Label the positive, negative, and partial charges on the atoms.
- In agricultural areas, farmers pay close attention to the weather forecast. Right before a predicted overnight freeze, farmers spray water on crops to protect the plants. Use the properties of water to explain how this



PRACTICE TEST
goo.gl/iAsVgI

method works. Be sure to mention why hydrogen bonds are responsible for this phenomenon.

- MAKE CONNECTIONS** What do climate change (see Concept 1.1 and Concept 3.2) and ocean acidification have in common?
- EVOLUTION CONNECTION** This chapter explains how the emergent properties of water contribute to the suitability of the environment for life. Until fairly recently, scientists assumed that other physical requirements for life included a moderate range of temperature, pH, atmospheric pressure, and salinity, as well as low levels of toxic chemicals. That view has changed with the discovery of organisms known as extremophiles, which have been found flourishing in hot, acidic sulfur springs, around hydrothermal vents deep in the ocean, and in soils with high levels of toxic metals. Why would astrobiologists be interested in studying extremophiles? What does the existence of life in such extreme environments say about the possibility of life on other planets?
- SCIENTIFIC INQUIRY** Design a controlled experiment to test the hypothesis that water acidification caused by acidic rain would inhibit the growth of *Elodea*, a freshwater plant (see Figure 2.17).
- WRITE ABOUT A THEME: ORGANIZATION** A sudden change in pH in a cell can be threatening to its survival. In a short essay (100–150 words), describe how a cell can resist pH changes.
- SYNTHESIZE YOUR KNOWLEDGE**



How do cats drink? Scientists using high-speed video have shown that cats use an interesting technique to drink aqueous substances like water and milk. Four times a second, the cat touches the tip of its tongue to the water and draws a column of water up into its mouth (as you can see in the photo), which then

shuts before gravity can pull the water back down. Describe how the properties of water allow cats to drink in this fashion, including how water's molecular structure contributes to the process.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Carbon: The Basis of Molecular Diversity

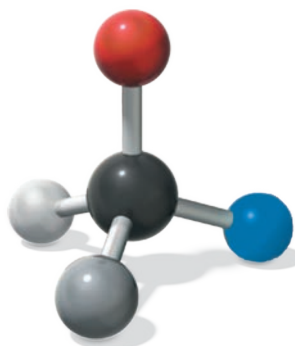
4



▲ **Figure 4.1** What properties make carbon the basis of all life?

KEY CONCEPTS

- 4.1** Organic chemistry is the study of carbon compounds
- 4.2** Carbon atoms can form diverse molecules by bonding to four other atoms
- 4.3** A few chemical groups are key to molecular function



▲ Carbon can bond to four other atoms or groups of atoms, making a large variety of molecules possible.

Carbon: The Backbone of Life

Living organisms, such as the plants and the African lion shown in **Figure 4.1**, are made up of chemicals based mostly on the element carbon. Carbon enters the biosphere through the action of producers—plants and other photosynthetic organisms that use solar energy to transform atmospheric CO_2 into the carbon-based molecules of life. These molecules are then taken up by consumers, which feed on other organisms.

Of all the chemical elements, carbon is unparalleled in its ability to form molecules that are large, complex, and varied, making possible the diversity of organisms that have evolved on Earth. Proteins, DNA, carbohydrates, and other molecules that distinguish living matter from inanimate material are all composed of carbon atoms bonded to one another and to atoms of other elements. Hydrogen (H), oxygen (O), nitrogen (N), sulfur (S), and phosphorus (P) are other common ingredients of these compounds, but it is the element carbon (C) that accounts for the enormous variety of biological molecules.

Large biological molecules, such as proteins, are the main focus of Chapter 5. In this chapter, we investigate the properties of smaller molecules. We will use these small molecules to illustrate concepts of molecular architecture that help explain why carbon is so important to life, while highlighting the theme that emergent properties arise from the organization of matter in living organisms.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

CONCEPT 4.1

Organic chemistry is the study of carbon compounds

For historical reasons, compounds containing carbon are said to be organic, and their study is called **organic chemistry**. By the early 1800s, chemists had learned to make simple compounds in the laboratory by combining elements under the right conditions. Artificial synthesis of the complex molecules extracted from living matter seemed impossible, however. Organic compounds were thought to arise only in living organisms, which were believed to contain a life force beyond the jurisdiction of physical and chemical laws.

Chemists began to chip away at this notion when they learned to synthesize organic compounds in the laboratory. In 1828, Friedrich Wöhler, a German chemist, tried to make an “inorganic” salt, ammonium cyanate, by mixing solutions of ammonium ions (NH_4^+) and cyanate ions (CNO^-). Wöhler was astonished to find that instead he had made urea, an organic compound present in the urine of animals.

The next few decades saw laboratory synthesis of increasingly complex organic compounds, supporting the view that physical and chemical laws govern the processes of life. Organic chemistry was redefined as the study of carbon compounds, regardless of origin. Organic compounds range from simple molecules, such as methane (CH_4), to colossal ones, such as proteins, with thousands of atoms.

Organic Molecules and the Origin of Life on Earth

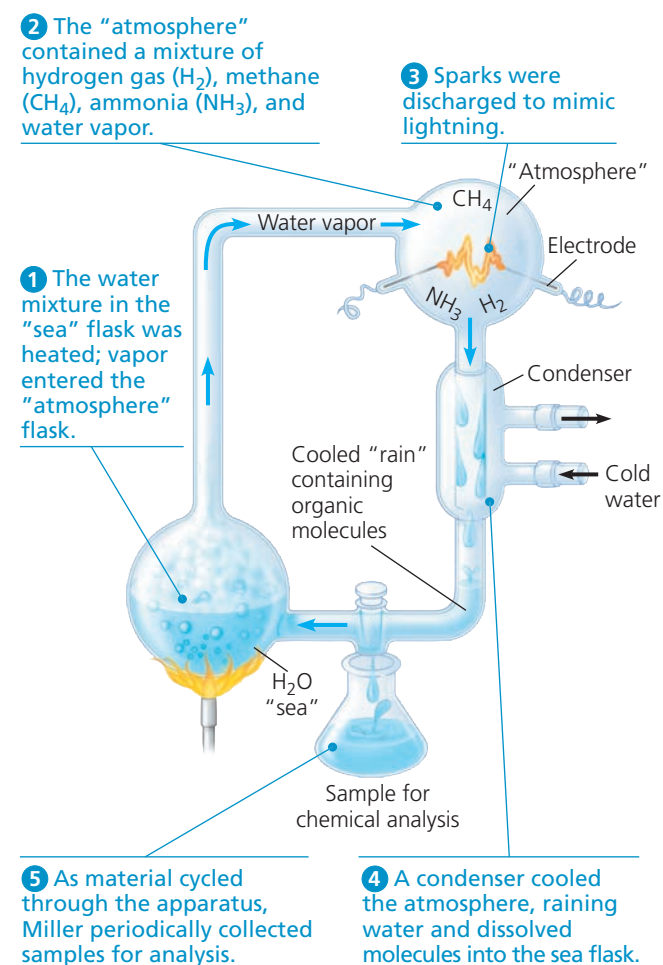
EVOLUTION In 1953, Stanley Miller, a graduate student of Harold Urey at the University of Chicago, helped bring the abiotic (nonliving) synthesis of organic compounds into the context of evolution. Study **Figure 4.2** to learn about his classic experiment. From his results, Miller concluded that complex organic molecules could arise spontaneously under conditions thought at that time to have existed on the early Earth. You can work with the data from a related experiment in the **Scientific Skills Exercise**. These experiments support the idea that abiotic synthesis of organic compounds, perhaps near volcanoes, could have been an early stage in the origin of life (see Figure 25.2).

The overall percentages of the major elements of life—C, H, O, N, S, and P—are quite uniform from one organism to another, reflecting the common evolutionary origin of all life. Because of carbon’s ability to form four bonds, however, this limited assortment of atomic building blocks can be used to build an inexhaustible variety of organic molecules. Different species of organisms, and different individuals within a species, are distinguished by variations in the types of organic molecules they make. In a sense, the great diversity

▼ **Figure 4.2**

Inquiry Can organic molecules form under conditions estimated to simulate those on the early Earth?

Experiment In 1953, Stanley Miller set up a closed system to mimic conditions thought at that time to have existed on the early Earth. A flask of water simulated the primeval sea. The water was heated so that some vaporized and moved into a second, higher flask containing the “atmosphere”—a mixture of gases. Sparks were discharged in the synthetic atmosphere to mimic lightning.



Results Miller identified a variety of organic molecules that are common in organisms. These included simple compounds, such as formaldehyde (CH_2O) and hydrogen cyanide (HCN), and more complex molecules, such as amino acids and long chains of carbon and hydrogen known as hydrocarbons.

Conclusion Organic molecules, a first step in the origin of life, may have been synthesized abiotically on the early Earth. Although new evidence indicates that the early Earth’s atmosphere was different from the “atmosphere” used by Miller in this experiment, recent experiments using the revised list of chemicals also produced organic molecules. (We will explore this hypothesis in more detail in Concept 25.1.)

Data from S. L. Miller, A production of amino acids under possible primitive Earth conditions, *Science* 117:528–529 (1953).

WHAT IF ► If Miller had increased the concentration of NH_3 in his experiment, how might the relative amounts of the products HCN and CH_2O have differed?



Interview with Stanley Miller: Investigating the origin of life

SCIENTIFIC SKILLS EXERCISE

Working with Moles and Molar Ratios

Could the First Biological Molecules Have Formed Near Volcanoes on Early Earth? In 2007, Jeffrey Bada, a former graduate student of Stanley Miller, discovered some vials of samples that had never been analyzed from an experiment performed by Miller in 1958. In that experiment, Miller used hydrogen sulfide gas (H_2S) as one of the gases in the reactant mixture. Since H_2S is released by volcanoes, the H_2S experiment was designed to mimic conditions near volcanoes on early Earth. In 2011, Bada and colleagues published the results of their analysis of these “lost” samples. In this exercise, you will make calculations using the molar ratios of reactants and products from the H_2S experiment.

How the Experiment Was Done According to his laboratory notebook, Miller used the same apparatus as in his original experiment (see Figure 4.2), but the mixture of gaseous reactants included methane (CH_4), carbon dioxide (CO_2), hydrogen sulfide (H_2S), and ammonia (NH_3). After three days of simulated volcanic activity, he collected samples of the liquid, partially purified the chemicals, and sealed the samples in sterile vials. In 2011, Bada’s research team used modern analytical methods to analyze the products in the vials for the presence of amino acids, the building blocks of proteins.

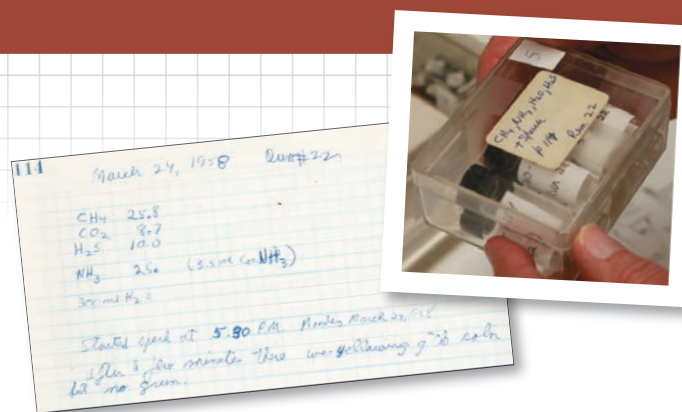
Data from the Experiment The table below shows 4 of the 23 amino acids detected in the 2011 analysis of the samples from Miller’s 1958 H_2S experiment.

Product Compound	Molecular Formula	Molar Ratio (Relative to Glycine)
Glycine	$\text{C}_2\text{H}_5\text{NO}_2$	1.0
Serine	$\text{C}_3\text{H}_7\text{NO}_3$	3.0×10^{-2}
Methionine	$\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$	1.8×10^{-3}
Alanine	$\text{C}_3\text{H}_7\text{NO}_2$	1.1

Data from E. T. Parker et al., Primordial synthesis of amines and amino acids in a 1958 Miller H_2S -rich spark discharge experiment, *Proceedings of the National Academy of Sciences USA* 108:5526–5531 (2011). www.pnas.org/cgi/doi/10.1073/pnas.1019191108.

INTERPRET THE DATA

1. A *mole* is the number of particles of a substance with a mass equivalent to its molecular (or atomic) mass in daltons. There are 6.02×10^{23} molecules (or atoms) in 1.0 mole (Avogadro’s number; see Concept 3.2). The data table shows the “molar ratios” of some of the products from the Miller H_2S experiment. In a molar ratio, each unitless value is expressed relative to a standard for



▲ Some of Stanley Miller’s notes from his 1958 hydrogen sulfide (H_2S) experiment along with his original vials.

that experiment. Here, the standard is the number of moles of the amino acid glycine, which is set to a value of 1.0. For instance, serine has a molar ratio of 3.0×10^{-2} , meaning that for every mole of glycine, there is 3.0×10^{-2} mole of serine. (a) Give the molar ratio of methionine to glycine and explain what it means. (b) How many molecules of glycine are present in 1.0 mole? (c) For every 1.0 mole of glycine in the sample, how many molecules of methionine are present? (Recall that to multiply two numbers with exponents, you add their exponents; to divide them, you subtract the exponent in the denominator from that in the numerator.)

2. (a) Which amino acid is present in higher amounts than glycine? (b) How many more molecules of that amino acid are present than the number of molecules in 1.0 mole of glycine?
3. The synthesis of products is limited by the amount of reactants. (a) If one mole each of CH_4 , NH_3 , H_2S , and CO_2 is added to 1 liter of water (= 55.5 moles of H_2O) in a flask, how many moles of hydrogen, carbon, oxygen, nitrogen, and sulfur are in the flask? (b) Looking at the molecular formula in the table, how many moles of each element would be needed to make 1.0 mole of glycine? (c) What is the maximum number of moles of glycine that could be made in that flask, with the specified ingredients, if no other molecules were made? Explain. (d) If serine or methionine were made individually, which element(s) would be used up first for each? How much of each product could be made?
4. The earlier published experiment carried out by Miller did not include H_2S in the reactants (see Figure 4.2). Which of the compounds shown in the data table can be made in the H_2S experiment but could not be made in the earlier experiment?

Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

of living organisms we see on the planet (and in fossil remains) is made possible by the unique chemical versatility of the element carbon.

CONCEPT CHECK 4.1

1. Why was Wöhler astonished to find he had made urea?
2. **VISUAL SKILLS** ▶ See Figure 4.2. Miller carried out a control experiment without discharging sparks and found no organic compounds. What might explain this result?

For suggested answers, see Appendix A.

CONCEPT 4.2

Carbon atoms can form diverse molecules by bonding to four other atoms

The key to an atom’s chemical characteristics is its electron configuration. This configuration determines the kinds and number of bonds an atom will form with other atoms. Recall that it is the valence electrons, those in

▼ **Figure 4.3** The shapes of three simple organic molecules.

Molecule and Molecular Shape	Molecular Formula	Structural Formula	Ball-and-Stick Model (molecular shape in pink)	Space-Filling Model
(a) Methane. When a carbon atom has four single bonds to other atoms, the molecule is tetrahedral.	CH_4	$ \begin{array}{c} \text{H} \\ \\ \text{H} - \text{C} - \text{H} \\ \\ \text{H} \end{array} $		
(b) Ethane. A molecule may have more than one tetrahedral group of single-bonded atoms. (Ethane consists of two such groups.)	C_2H_6	$ \begin{array}{c} \text{H} \quad \text{H} \\ \quad \\ \text{H} - \text{C} - \text{C} - \text{H} \\ \quad \\ \text{H} \quad \text{H} \end{array} $		
(c) Ethene (ethylene). When two carbon atoms are joined by a double bond, all atoms attached to those carbons are in the same plane, and the molecule is flat.	C_2H_4	$ \begin{array}{c} \text{H} \quad \text{H} \\ \backslash \quad / \\ \text{C} = \text{C} \\ / \quad \backslash \\ \text{H} \quad \text{H} \end{array} $		

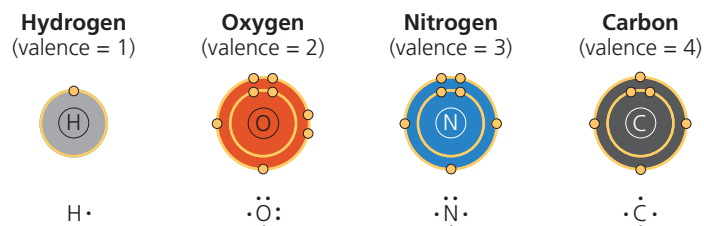
the outermost shell, that are available to form bonds with other atoms.

The Formation of Bonds with Carbon

Carbon has 6 electrons, with 2 in the first electron shell and 4 in the second shell; thus, it has 4 valence electrons in a shell that can hold up to 8 electrons. A carbon atom usually completes its valence shell by sharing its 4 electrons with other atoms so that 8 electrons are present. Each pair of shared electrons constitutes a covalent bond (see Figure 2.10d). In organic molecules, carbon usually forms single or double covalent bonds. Each carbon atom acts as an intersection point from which a molecule can branch off in as many as four directions. This enables carbon to form large, complex molecules.

When a carbon atom forms four single covalent bonds, the arrangement of its four hybrid orbitals causes the bonds to angle toward the corners of an imaginary tetrahedron. The bond angles in methane (CH_4) are 109.5° (Figure 4.3a), and they are roughly the same in any group of atoms where carbon has four single bonds. For example, ethane (C_2H_6) is shaped like two overlapping tetrahedrons (Figure 4.3b). In molecules with more carbons, every grouping of a carbon bonded to four other atoms has a tetrahedral shape. But when two carbon atoms are joined by a double bond, as in ethene (C_2H_4), the bonds from both carbons are all in the same plane, so the atoms joined to those carbons are in the same plane as well (Figure 4.3c). We find it convenient to write molecules as structural formulas, as if the molecules being represented are two-dimensional,

▼ **Figure 4.4** Valences of the major elements of organic molecules. Valence, the number of covalent bonds an atom can form, is generally equal to the number of electrons required to complete the valence shell. All electrons are shown in the electron distribution diagrams (top), but only valence shell electrons are shown in the Lewis dot structures (bottom). Note that carbon can form four bonds.



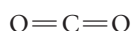
MAKE CONNECTIONS ► Draw the Lewis dot structures for sodium, phosphorus, sulfur, and chlorine. (Refer to Figure 2.7.)

but keep in mind that molecules are three-dimensional and that the shape of a molecule is central to its function.

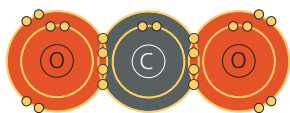
The number of unpaired electrons in the valence shell of an atom is generally equal to the atom's **valence**, the number of covalent bonds it can form. Figure 4.4 shows the valences of carbon and its most frequent bonding partners—hydrogen, oxygen, and nitrogen. These are the four main atoms in organic molecules.

The electron configuration of carbon gives it covalent compatibility with many different elements. Let's consider how valence and the rules of covalent bonding apply to carbon atoms with partners other than hydrogen. We'll look at two examples, the simple molecules carbon dioxide and urea.

In the carbon dioxide molecule (CO_2), a single carbon atom is joined to two atoms of oxygen by double covalent bonds. The structural formula for CO_2 is shown here:

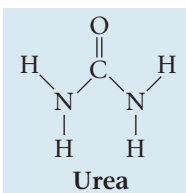


Each line in a structural formula represents a pair of shared electrons. Thus, the two double bonds in CO_2 have the same number of shared electrons as four single bonds. The arrangement completes the valence shells of all atoms in the molecule:



Because CO_2 is a very simple molecule and lacks hydrogen, it is often considered inorganic, even though it contains carbon. Whether we call CO_2 organic or inorganic, however, it is clearly important to the living world as the source of carbon, via photosynthetic organisms, for all organic molecules in organisms (see Concept 2.4).

Urea, $\text{CO}(\text{NH}_2)_2$, is the organic compound found in urine that Wöhler synthesized in the early 1800s. Again, each atom has the required number of covalent bonds. In this case, one carbon atom participates in both single and double bonds.



Urea and carbon dioxide are molecules with only one carbon atom. But as Figure 4.3 shows, a carbon atom can also use one or more valence electrons to form covalent bonds to other carbon atoms, each of which can also form four bonds. Thus, the atoms can be linked into chains of seemingly infinite variety.

Molecular Diversity Arising from Variation in Carbon Skeletons

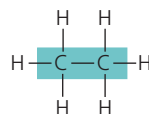
Carbon chains form the skeletons of most organic molecules. The skeletons vary in length and may be straight, branched, or arranged in closed rings (Figure 4.5). Some carbon skeletons have double bonds, which vary in number and location. Such variation in carbon skeletons is one important source of the molecular complexity and diversity that characterize living matter. In addition, atoms of other elements can be bonded to the skeletons at available sites.

Hydrocarbons

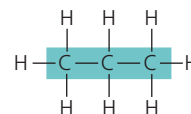
All of the molecules that are shown in Figures 4.3 and 4.5 are **hydrocarbons**, organic molecules consisting of only carbon and hydrogen. Atoms of hydrogen are attached to the carbon skeleton wherever electrons are available for covalent bonding. Hydrocarbons are the major components of petroleum, which is called a fossil fuel because it consists of the partially decomposed remains of organisms that lived millions of years ago.

▼ **Figure 4.5** Four ways that carbon skeletons can vary.

(a) Length



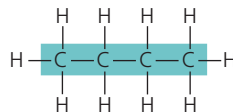
Ethane



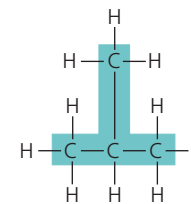
Propane

Carbon skeletons vary in length.

(b) Branching



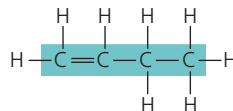
Butane



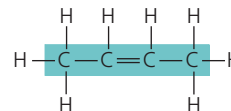
2-Methylpropane
(commonly called isobutane)

Skeletons may be unbranched or branched.

(c) Double bond position



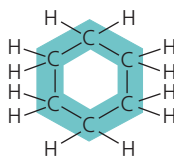
1-Butene



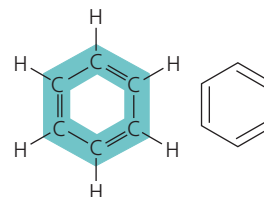
2-Butene

The skeleton may have double bonds, which can vary in location.

(d) Presence of rings



Cyclohexane



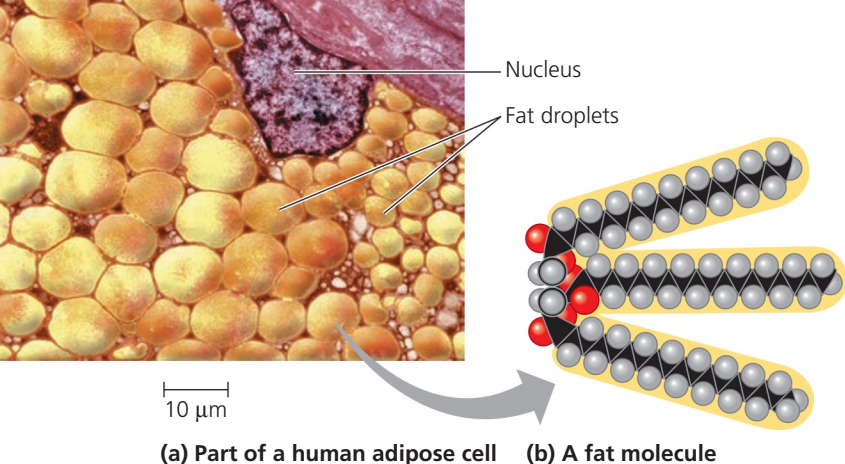
Benzene

Some carbon skeletons are arranged in rings. In the abbreviated structural formula for each compound (to its right), each corner represents a carbon and its attached hydrogens.



Animation: Diversity of Carbon-Based Molecules

Although hydrocarbons are not prevalent in most living organisms, many of a cell's organic molecules have regions consisting of only carbon and hydrogen. For example, the molecules known as fats have long hydrocarbon tails attached to a nonhydrocarbon component (Figure 4.6). Neither petroleum nor fat dissolves in water; both are hydrophobic compounds because the great majority of their bonds are relatively nonpolar carbon-to-hydrogen linkages. Another characteristic of hydrocarbons is that they can undergo reactions that release a relatively large amount of energy. The gasoline that fuels a car consists of hydrocarbons, and the hydrocarbon tails of fats serve as stored fuel for plant embryos (seeds) and animals.



▲ Figure 4.6 The role of hydrocarbons in fats.

(a) Mammalian adipose cells stockpile fat molecules as a fuel reserve. This colorized micrograph shows part of a human adipose cell with many fat droplets, each containing a large number of fat molecules. **(b)** A fat molecule consists of a small, nonhydrocarbon component joined to three hydrocarbon tails that account for the hydrophobic behavior of fats. The tails can be broken down to provide energy. (Black = carbon; gray = hydrogen; red = oxygen.)

MAKE CONNECTIONS ► How do the tails account for the hydrophobic nature of fats? (See Concept 3.2.)

Isomers

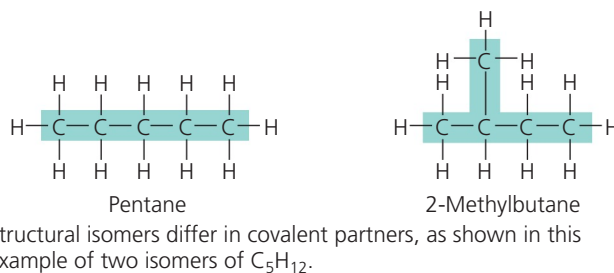
Variation in the architecture of organic molecules can be seen in **isomers**, compounds that have the same numbers of atoms of the same elements but different structures and hence different properties. We will examine three types of isomers: structural isomers, *cis-trans* isomers, and enantiomers.

Structural isomers differ in the covalent arrangements of their atoms. Compare, for example, the two five-carbon compounds in **Figure 4.7a**. Both have the molecular formula C_5H_{12} , but they differ in the covalent arrangement of their carbon skeletons. The skeleton is straight in one compound but branched in the other. The number of possible isomers increases tremendously as carbon skeletons increase in size. There are only three forms of C_5H_{12} (two of which are shown in Figure 4.7a), but there are 18 variants of C_8H_{18} and 366,319 possible structural isomers of $C_{20}H_{42}$. Structural isomers may also differ in the location of double bonds.

In ***cis-trans* isomers**, carbons have covalent bonds to the same atoms, but these atoms differ in their spatial arrangements due to the inflexibility of double bonds. Single bonds allow the atoms they join to rotate freely about the bond axis without changing the compound. In contrast, double bonds do not permit such rotation. If a double bond joins two carbon atoms, and each C also has two different atoms (or groups of atoms) attached to it, then two distinct *cis-trans* isomers are possible. Consider a simple molecule with two double-bonded carbons, each of which has an H and an X attached to it (**Figure 4.7b**). The arrangement with both Xs on the same side of the double bond is called a *cis isomer*, and that with the Xs on opposite sides is called a *trans isomer*. The subtle difference in shape between such isomers can have a

▼ Figure 4.7 Three types of isomers. Isomers are compounds that have the same molecular formula but different structures.

(a) Structural isomers



(b) *Cis-trans* isomers

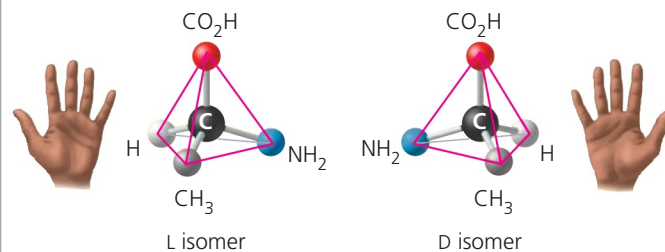


cis isomer: The two Xs are on the same side.

trans isomer: The two Xs are on opposite sides.

Cis-trans isomers differ in arrangement about a double bond. In these diagrams, X represents an atom or group of atoms attached to a double-bonded carbon.

(c) Enantiomers



Enantiomers differ in spatial arrangement around an asymmetric carbon, resulting in molecules that are mirror images, like left and right hands. The two isomers here are designated the L and D isomers from the Latin for "left" and "right" (*levo* and *dextro*). Enantiomers cannot be superimposed on each other.

DRAW IT ► There are three structural isomers of C_5H_{12} ; draw the one not shown in (a).



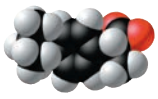
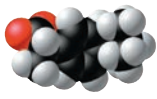
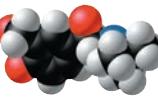
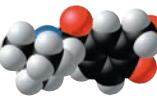
Animation: Isomers

dramatic effect on the biological activities of organic molecules. For example, the biochemistry of vision involves a light-induced change of retinal, a chemical compound in the eye, from the *cis* isomer to the *trans* isomer (see Figure 50.17). Another example involves *trans* fats, harmful fats formed during food processing that are discussed in Concept 5.3.

Enantiomers are isomers that are mirror images of each other and that differ in shape due to the presence of an *asymmetric carbon*, one that is attached to four different atoms or groups of atoms. (See the middle carbon in the ball-and-stick models shown in **Figure 4.7c**.) The four groups can

▼ Figure 4.8 The pharmacological importance of enantiomers.

Ibuprofen and albuterol are drugs whose enantiomers have different effects. (*S* and *R* are used here to distinguish between enantiomers.) Ibuprofen is commonly sold as a mixture of the two enantiomers; the *S* enantiomer is 100 times more effective than the *R* form. Albuterol is synthesized and sold only as the *R* form of that particular drug; the *S* form counteracts the active *R* form.

Drug	Effects	Effective Enantiomer	Ineffective Enantiomer
Ibuprofen	Reduces inflammation and pain	 <i>S</i> -Ibuprofen	 <i>R</i> -Ibuprofen
Albuterol	Relaxes bronchial (airway) muscles, improving airflow in asthma patients	 <i>R</i> -Albuterol	 <i>S</i> -Albuterol

be arranged in space around the asymmetric carbon in two different ways that are mirror images. Enantiomers are, in a way, left-handed and right-handed versions of the molecule. Just as your right hand won't fit into a left-handed glove, a "right-handed" molecule won't fit into the same space as the "left-handed" version. Usually, only one isomer is biologically active because only that form can bind to specific molecules in an organism.

The concept of enantiomers is important in the pharmaceutical industry because the two enantiomers of a drug may not be equally effective, as is the case for both ibuprofen and the asthma medication albuterol (Figure 4.8). Methamphetamine also occurs in two enantiomers that have very different effects. One enantiomer is the highly addictive stimulant drug known as "crank," sold illegally in the street drug trade. The other has a much weaker effect and is the active ingredient in an over-the-counter vapor inhaler for treatment of nasal congestion. The differing effects of enantiomers in the body demonstrate that organisms are sensitive to even the subtlest variations in molecular architecture. Once again, we see that molecules have emergent properties that depend on the specific arrangement of their atoms.

CONCEPT CHECK 4.2

- DRAW IT** > (a) Draw a structural formula for C_2H_4 .
(b) Draw the *trans* isomer of $C_2H_2Cl_2$.
- VISUAL SKILLS** > Which two pairs of molecules in Figure 4.5 are isomers? For each pair, identify the type of isomer.
- How are gasoline and fat chemically similar?
- VISUAL SKILLS** > See Figures 4.5a and 4.7. Can propane (C_3H_8) form isomers? Explain.

For suggested answers, see Appendix A.

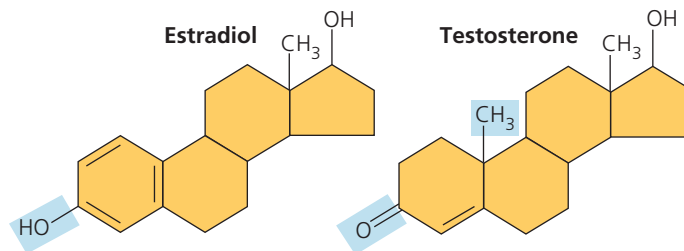
CONCEPT 4.3

A few chemical groups are key to molecular function

The properties of an organic molecule depend not only on the arrangement of its carbon skeleton but also on the various chemical groups attached to that skeleton. We can think of hydrocarbons, the simplest organic molecules, as the underlying framework for more complex organic molecules. A number of chemical groups can replace one or more hydrogens of the hydrocarbon. These groups may participate in chemical reactions or may contribute to function indirectly by their effects on molecular shape; they help give each molecule its unique properties.

The Chemical Groups Most Important in the Processes of Life

Consider the differences between estradiol (a type of estrogen) and testosterone. These compounds are female and male sex hormones, respectively, in humans and other vertebrates. Both are steroids, organic molecules with a common carbon skeleton in the form of four fused rings. They differ only in the chemical groups attached to the rings (shown here in abbreviated form); the distinctions in molecular architecture are shaded in blue:

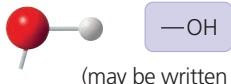
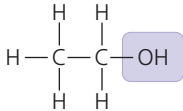
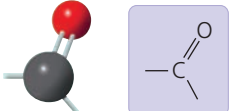
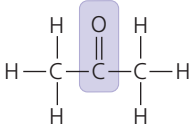
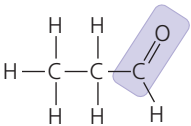
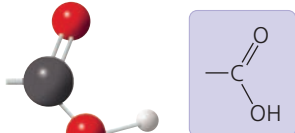
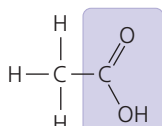
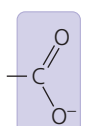
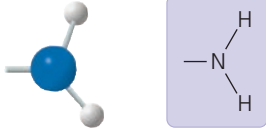
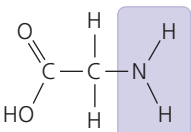
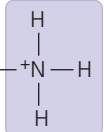
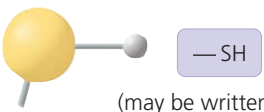
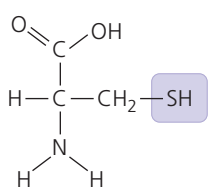
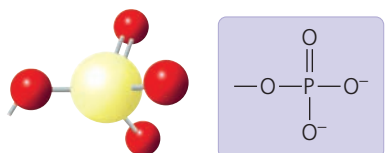
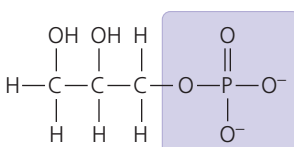
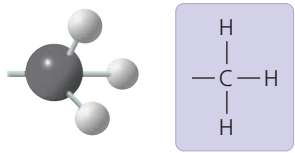
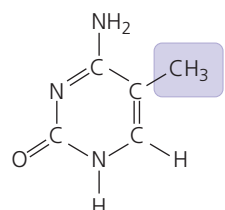


The different actions of these two molecules on many targets throughout the body are the basis of gender, producing the contrasting features of male and female vertebrates. In this case, the chemical groups are important because they affect molecular shape, contributing to function.

In other cases, chemical groups are directly involved in chemical reactions; such groups are known as **functional groups**. Each has certain properties, such as shape and charge, that cause it to participate in chemical reactions in a characteristic way.

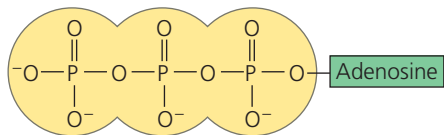
The seven chemical groups most important in biological processes are the hydroxyl, carbonyl, carboxyl, amino, sulfhydryl, phosphate, and methyl groups. The first six groups can be chemically reactive; of these six, all except the sulfhydryl group are also hydrophilic and thus increase the solubility of organic compounds in water. The methyl group is not reactive, but instead often serves as a recognizable tag on biological molecules. Study Figure 4.9 to become familiar with these biologically important chemical groups.

▼ **Figure 4.9** Some biologically important chemical groups.

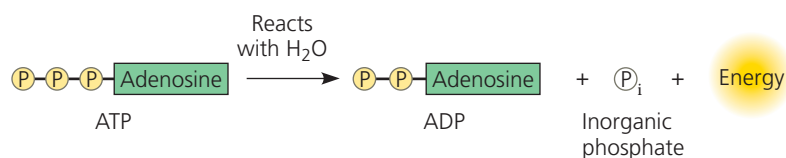
Chemical Group	Group Properties and Compound Name	Examples
Hydroxyl group (—OH)  (may be written HO—)	Is polar due to electronegative oxygen. Forms hydrogen bonds with water, helping dissolve compounds such as sugars. Compound name: Alcohol (specific name usually ends in <i>-ol</i>)	 Ethanol , the alcohol present in alcoholic beverages
Carbonyl group (>C=O) 	Sugars with ketone groups are called ketoses; those with aldehydes are called aldoses. Compound name: Ketone (carbonyl group is within a carbon skeleton) or aldehyde (carbonyl group is at the end of a carbon skeleton)	 Acetone , the simplest ketone  Propanal , an aldehyde
Carboxyl group (—COOH) 	Acts as an acid (can donate H^+) because the covalent bond between oxygen and hydrogen is so polar. Compound name: Carboxylic acid , or organic acid	 $\rightleftharpoons$  + H^+ Acetic acid , which gives vinegar its sour taste Ionized form of —COOH (carboxylate ion), found in cells
Amino group (—NH_2) 	Acts as a base; can pick up an H^+ from the surrounding solution (water, in living organisms). Compound name: Amine	 + H^+ $\rightleftharpoons$  Glycine , an amino acid (note its carboxyl group) Ionized form of —NH_2 , found in cells
Sulfhydryl group (—SH)  (may be written HS—)	Two —SH groups can react, forming a “cross-link” that helps stabilize protein structure. Hair protein cross-links maintain the straightness or curliness of hair; in hair salons, permanent treatments break cross-links, then re-form them while the hair is in the desired shape. Compound name: Thiol	 Cysteine , a sulfur-containing amino acid
Phosphate group (—OPO_3^{2-}) 	Contributes negative charge (1– when positioned inside a chain of phosphates; 2– when at the end). When attached, confers on a molecule the ability to react with water, releasing energy. Compound name: Organic phosphate	 Glycerol phosphate , which takes part in many important chemical reactions in cells
Methyl group (—CH_3) 	Affects the expression of genes when on DNA or on proteins bound to DNA. Affects the shape and function of male and female sex hormones. Compound name: Methylated compound	 5-Methylcytosine , a component of DNA that has been modified by addition of a methyl group

ATP: An Important Source of Energy for Cellular Processes

The “Phosphate group” row in Figure 4.9 shows a simple example of an organic phosphate molecule. A more complicated organic phosphate, **adenosine triphosphate**, or **ATP**, is worth mentioning here because its function in the cell is so important. ATP consists of an organic molecule called adenosine attached to a string of three phosphate groups:



Where three phosphates are present in series, as in ATP, one phosphate may be split off as a result of a reaction with water. This inorganic phosphate ion, HOPO_3^{2-} , is often abbreviated P_i in this book, and a phosphate group in an organic molecule is often written as P . Having lost one phosphate, ATP becomes adenosine *diphosphate*, or ADP. Although ATP is sometimes said to store energy, it is more accurate to think of it as storing the potential to react with water. This reaction releases energy that can be used by the cell. You will learn about this in more detail in Concept 6.3.



The Chemical Elements of Life: A Review

Living matter, as you have learned, consists mainly of carbon, oxygen, hydrogen, and nitrogen, with smaller amounts of sulfur and phosphorus. These elements all form strong covalent bonds, an essential characteristic in the architecture of complex organic molecules. Of all these elements, carbon is the virtuoso of the covalent bond. The versatility of carbon makes possible the great diversity of organic molecules, each with particular properties that emerge from the unique arrangement of its carbon skeleton and the chemical groups appended to that skeleton. This variation at the molecular level provides the foundation for the rich biological diversity found on our planet.

CONCEPT CHECK 4.3

- VISUAL SKILLS** > What does the term *amino acid* signify about the structure of such a molecule? See Figure 4.9.
- What chemical change occurs to ATP when it reacts with water and releases energy?
- DRAW IT** > Suppose you had an organic molecule such as cysteine (see Figure 4.9, sulfhydryl group example), and you chemically removed the —NH_2 group and replaced it with —COOH . Draw this structure. How would this change the chemical properties of the molecule? Is the central carbon asymmetric before the change? After?

For suggested answers, see Appendix A.

4 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 4.1

Organic chemistry is the study of carbon compounds (pp. 105–106)

- Organic** compounds, once thought to arise only within living organisms, were finally synthesized in the laboratory.
- Living matter is made mostly of carbon, oxygen, hydrogen, and nitrogen. Biological diversity results from carbon's ability to form a huge number of molecules with particular shapes and properties.



How did Stanley Miller's experiments support the idea that, even at life's origins, physical and chemical laws govern the processes of life?



CONCEPT 4.2

Carbon atoms can form diverse molecules by bonding to four other atoms (pp. 106–110)

- Carbon, with a valence of 4, can bond to various other atoms, including O, H, and N. Carbon can also bond to other carbon



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atoms, forming the carbon skeletons of organic compounds. These skeletons vary in length and shape and have bonding sites for atoms of other elements.

- Hydrocarbons** consist of carbon and hydrogen.
- Isomers** are compounds that have the same molecular formula but different structures and therefore different properties. Three types of isomers are **structural isomers**, **cis-trans isomers**, and **enantiomers**.

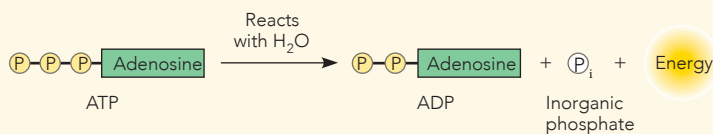
VISUAL SKILLS > Refer back to Figure 4.9. What type of isomers are acetone and propanal? How many asymmetric carbons are present in acetic acid, glycine, and glycerol phosphate? Can these three molecules exist as forms that are enantiomers?

CONCEPT 4.3

A few chemical groups are key to molecular function (pp. 110–112)

- Chemical groups attached to the carbon skeletons of organic molecules participate in chemical reactions (**functional groups**) or contribute to function by affecting molecular shape (see Figure 4.9).
- ATP (adenosine triphosphate)** consists of adenosine attached to three phosphate groups. ATP can react with water, forming

ADP (adenosine diphosphate) and inorganic phosphate. This reaction releases energy that can be used by the cell.



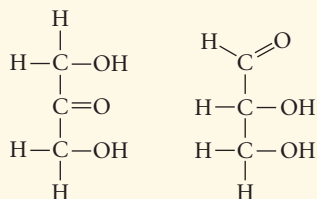
- 2 In what ways does a methyl group differ chemically from the other six important chemical groups shown in Figure 4.9?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

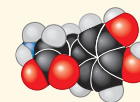
8. Which of the following molecules has an asymmetric carbon? Which carbon is asymmetric?



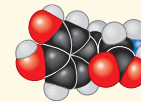
PRACTICE TEST
goo.gl/iAsVgI

9. **EVOLUTION CONNECTION • DRAW IT** Some scientists think that life elsewhere in the universe might be based on the element silicon, rather than on carbon, as on Earth. Look at the electron distribution diagram for silicon in Figure 2.7 and draw the Lewis dot structure for silicon. What properties does silicon share with carbon that would make silicon-based life more likely than, say, neon-based life or aluminum-based life?
10. **SCIENTIFIC INQUIRY** 50 years ago, pregnant women who were prescribed thalidomide for morning sickness gave birth to children with birth defects. Thalidomide is a mixture of two enantiomers; one reduces morning sickness, but the other causes severe birth defects. Today, the FDA has approved this drug for non-pregnant individuals with Hansen's disease (leprosy) or newly diagnosed multiple myeloma, a blood and bone marrow cancer. The beneficial enantiomer can be synthesized and given to patients, but over time, both the beneficial *and* the harmful enantiomer can be detected in the body. Propose a possible explanation for the presence of the harmful enantiomer.

11. **WRITE ABOUT A THEME: ORGANIZATION** In 1918, an epidemic of sleeping sickness caused an unusual rigid paralysis in some survivors, similar to symptoms of advanced Parkinson's disease. Years later, L-dopa (below, left), a chemical used to treat Parkinson's disease, was given to some of these patients. L-dopa was remarkably effective at eliminating the paralysis, at least temporarily. However, its enantiomer, D-dopa (right), was subsequently shown to have no effect at all, as is the case for Parkinson's disease. In a short essay (100–150 words), discuss how the effectiveness of one enantiomer and not the other illustrates the theme of structure and function.



L-dopa



D-dopa

12. **SYNTHESIZE YOUR KNOWLEDGE**



Explain how the chemical structure of the carbon atom accounts for the differences between the male and female lions seen in the photo.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Biological Macromolecules and Lipids

5



▲ **Figure 5.1** Why is the structure of a protein important for its function?

KEY CONCEPTS

- 5.1** Macromolecules are polymers, built from monomers
- 5.2** Carbohydrates serve as fuel and building material
- 5.3** Lipids are a diverse group of hydrophobic molecules
- 5.4** Proteins include a diversity of structures, resulting in a wide range of functions
- 5.5** Nucleic acids store, transmit, and help express hereditary information
- 5.6** Genomics and proteomics have transformed biological inquiry and applications

The Molecules of Life

Given the rich complexity of life on Earth, it might surprise you that the most important large molecules found in all living things—from bacteria to elephants—can be sorted into just four main classes: carbohydrates, lipids, proteins, and nucleic acids. On the molecular scale, members of three of these classes—carbohydrates, proteins, and nucleic acids—are huge and are therefore called **macromolecules**. For example, a protein may consist of thousands of atoms that form a molecular colossus with a mass well over 100,000 daltons. Considering the size and complexity of macromolecules, it is noteworthy that biochemists have determined the detailed structure of so many of them. The image in **Figure 5.1** is a molecular model of a protein called alcohol dehydrogenase, which breaks down alcohol in the body.

The architecture of a large biological molecule plays an essential role in its function. Like water and simple organic molecules, large biological molecules exhibit unique emergent properties arising from the orderly arrangement of their atoms. In this chapter, we'll first consider how macromolecules are built. Then we'll examine the structure and function of all four classes of large biological molecules: carbohydrates, lipids, proteins, and nucleic acids.

◀ The scientist in the foreground is using 3-D glasses to help her visualize the structure of the protein displayed on her screen.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter



CONCEPT 5.1

Macromolecules are polymers, built from monomers

Large carbohydrates, proteins, and nucleic acids are chain-like molecules called polymers (from the Greek *polys*, many, and *meros*, part). A **polymer** is a long molecule consisting of many similar or identical building blocks linked by covalent bonds, much as a train consists of a chain of cars. The repeating units that serve as the building blocks of a polymer are smaller molecules called **monomers** (from the Greek *monos*, single). In addition to forming polymers, some monomers have functions of their own.

The Synthesis and Breakdown of Polymers

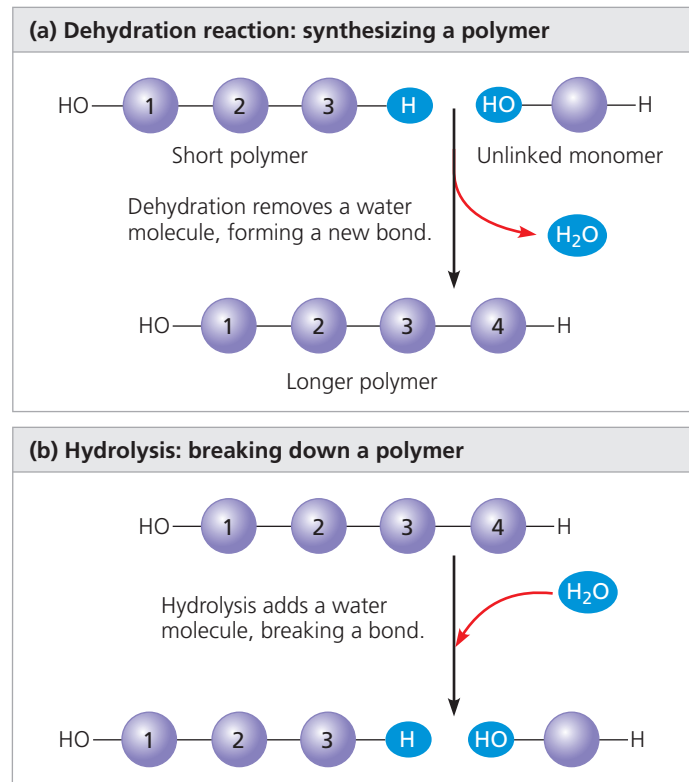
Although each class of polymer is made up of a different type of monomer, the chemical mechanisms by which cells make and break down polymers are basically the same in all cases. In cells, these processes are facilitated by **enzymes**, specialized macromolecules that speed up chemical reactions. The reaction connecting monomers is a good example of a **dehydration reaction**, a reaction in which two molecules are covalently bonded to each other with the loss of a water molecule (Figure 5.2a). When a bond forms between two monomers, each monomer contributes part of the water molecule that is released during the reaction: One monomer provides a hydroxyl group (—OH), while the other provides a hydrogen (—H). This reaction is repeated as monomers are added to the chain one by one, making a polymer (also called polymerization).

Polymers are disassembled to monomers by **hydrolysis**, a process that is essentially the reverse of the dehydration reaction (Figure 5.2b). Hydrolysis means water breakage (from the Greek *hydro*, water, and *lysis*, break). The bond between monomers is broken by the addition of a water molecule, with a hydrogen from water attaching to one monomer and the hydroxyl group attaching to the other. An example of hydrolysis within our bodies is the process of digestion. The bulk of the organic material in our food is in the form of polymers that are much too large to enter our cells. Within the digestive tract, various enzymes attack the polymers, speeding up hydrolysis. Released monomers are then absorbed into the bloodstream for distribution to all body cells. Those cells can then use dehydration reactions to assemble the monomers into new, different polymers that can perform specific functions required by the cell. (Dehydration reactions and hydrolysis can also be involved in the formation and breakdown of molecules that are not polymers, such as some lipids.)

The Diversity of Polymers

A cell has thousands of different macromolecules; the collection varies from one type of cell to another. The inherited

✓ Figure 5.2 The synthesis and breakdown of polymers.



Animation: Making and Breaking Polymers

differences between close relatives, such as human siblings, reflect small variations in polymers, particularly DNA and proteins. Molecular differences between unrelated individuals are more extensive, and those between species greater still. The diversity of macromolecules in the living world is vast, and the possible variety is effectively limitless.

What is the basis for such diversity in life's polymers? These molecules are constructed from only 40 to 50 common monomers and some others that occur rarely. Building a huge variety of polymers from such a limited number of monomers is analogous to constructing hundreds of thousands of words from only 26 letters of the alphabet. The key is arrangement—the particular linear sequence that the units follow. However, this analogy falls far short of describing the great diversity of macromolecules because most biological polymers have many more monomers than the number of letters in even the longest word. Proteins, for example, are built from 20 kinds of amino acids arranged in chains that are typically hundreds of amino acids long. The molecular logic of life is simple but elegant: Small molecules common to all organisms act as building blocks that are ordered into unique macromolecules.

Despite this immense diversity, molecular structure and function can still be grouped roughly by class. Let's examine each of the four major classes of large biological molecules. For each class, the large molecules have emergent properties not found in their individual components.

CONCEPT CHECK 5.1

1. What are the four main classes of large biological molecules? Which class does not consist of polymers?
2. How many molecules of water will be released if three monomers combine to form a polymer?
3. **WHAT IF? >** If you eat a piece of fish, what reactions must occur for the amino acid monomers in the protein of the fish to be converted to new proteins in your body?

For suggested answers, see Appendix A.

CONCEPT 5.2

Carbohydrates serve as fuel and building material

Carbohydrates include sugars and polymers of sugars. The simplest carbohydrates are the monosaccharides, or simple sugars; these are the monomers from which more complex carbohydrates are built. Disaccharides are double sugars, consisting of two monosaccharides joined by a covalent bond. Carbohydrate macromolecules are polymers called polysaccharides, composed of many sugar building blocks.



Animation: Carbohydrates

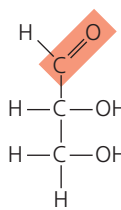
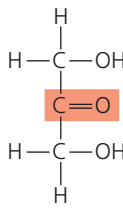
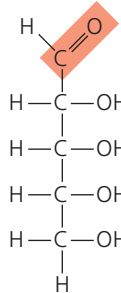
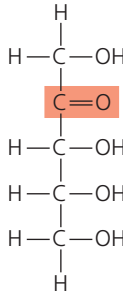
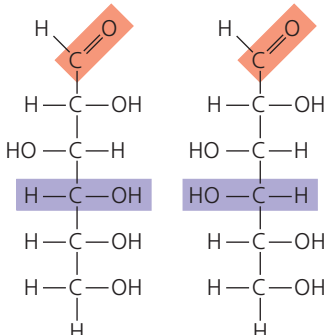
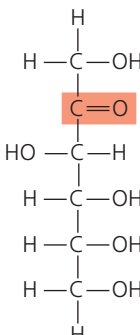
Sugars

Monosaccharides (from the Greek *monos*, single, and *sacchar*, sugar) generally have molecular formulas that are some multiple of the unit CH_2O . Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$), the most common monosaccharide, is of central importance in the chemistry of life. In the structure of glucose, we can see the trademarks of a sugar: The molecule has a carbonyl group, >C=O , and multiple hydroxyl groups, —OH (Figure 5.3). Depending on the location of the carbonyl group, a sugar is either an aldose (aldehyde sugar) or a ketose (ketone sugar). Glucose, for example, is an aldose; fructose, an isomer of glucose, is a ketose. (Most names for sugars end in *-ose*.) Another criterion for classifying sugars is the size of the carbon skeleton, which ranges from three to seven carbons long. Glucose, fructose, and other sugars that have six carbons are called hexoses. Trioses (three-carbon sugars) and pentoses (five-carbon sugars) are also common.

Still another source of diversity for simple sugars is in the way their parts are arranged spatially around asymmetric carbons. (Recall that an asymmetric carbon is a carbon attached to four different atoms or groups of atoms.) Glucose and galactose, for example, differ only in the placement of parts around one asymmetric carbon (see the purple boxes in Figure 5.3). What seems like a small difference is significant enough to give the two sugars distinctive shapes and binding activities, thus different behaviors.

Although it is convenient to draw glucose with a linear carbon skeleton, this representation is not completely accurate. In aqueous solutions, glucose molecules, as well as most other

Figure 5.3 The structure and classification of some monosaccharides. Sugars vary in the location of their carbonyl groups (orange), the length of their carbon skeletons, and the way their parts are arranged spatially around asymmetric carbons (compare, for example, the purple portions of glucose and galactose).

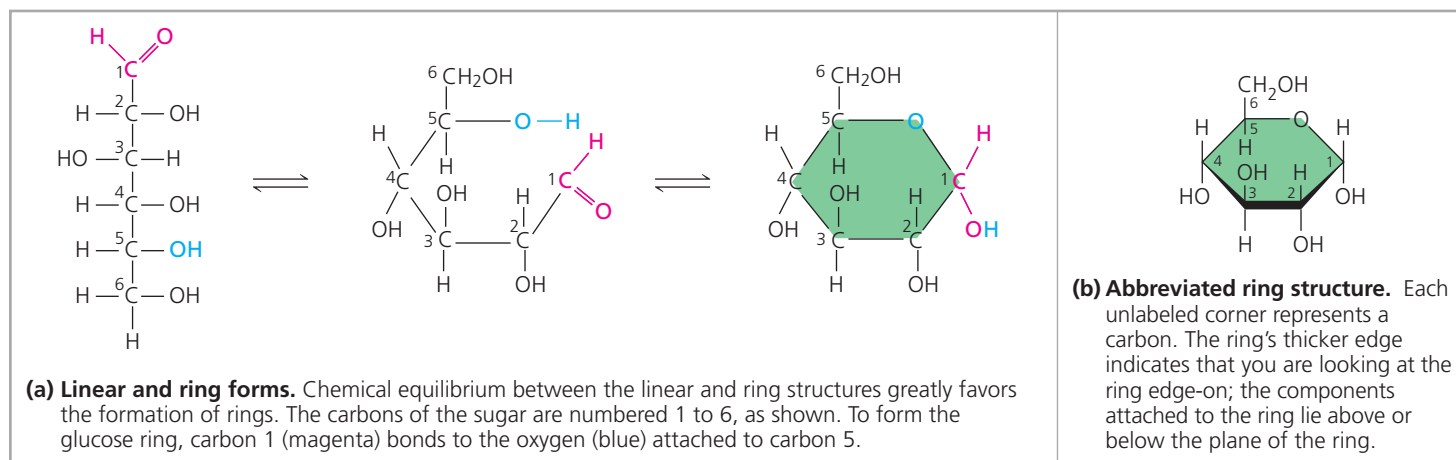
Aldoses (Aldehyde Sugars) Carbonyl group at end of carbon skeleton		Ketoses (Ketone Sugars) Carbonyl group within carbon skeleton	
Trioses: three-carbon sugars (C ₃ H ₆ O ₃)			
			
Glyceraldehyde An initial breakdown product of glucose		Dihydroxyacetone An initial breakdown product of glucose	
Pentoses: five-carbon sugars (C ₅ H ₁₀ O ₅)			
			
Ribose A component of RNA		Ribulose An intermediate in photosynthesis	
Hexoses: six-carbon sugars (C ₆ H ₁₂ O ₆)			
			
Glucose Energy sources for organisms		Galactose Energy sources for organisms	
		Fructose An energy source for organisms	

MAKE CONNECTIONS > In the 1970s, a process was developed that converts the glucose in corn syrup to its sweeter-tasting isomer, fructose. High-fructose corn syrup, a common ingredient in soft drinks and processed food, is a mixture of glucose and fructose. What type of isomers are glucose and fructose? (See Figure 4.7.)



Animation: Monosaccharides

▼ **Figure 5.4** Linear and ring forms of glucose.



DRAW IT ► Start with the linear form of fructose (see Figure 5.3) and draw the formation of the fructose ring in two steps, as shown in (a). First, number the carbons starting at the top of the linear structure. Then draw the molecule in a ringlike orientation, attaching carbon 5 via its oxygen to carbon 2. Compare the number of carbons in the fructose and glucose rings.

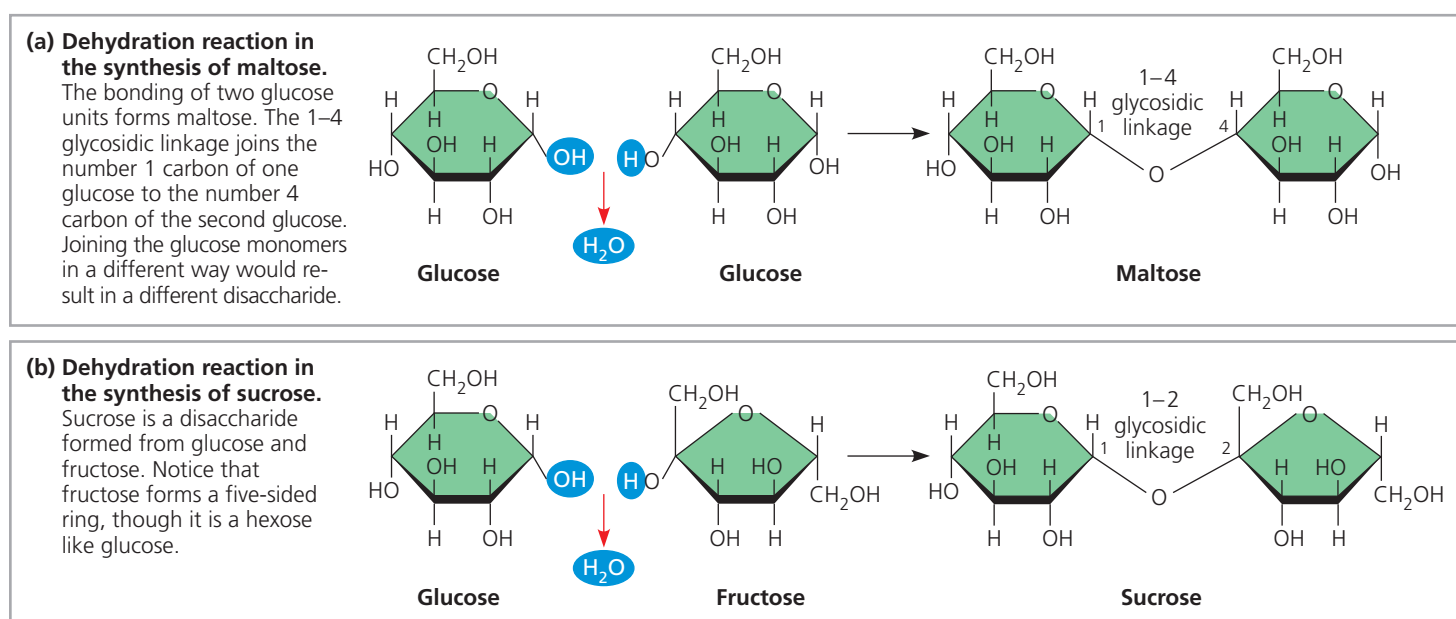
five- and six-carbon sugars, form rings, because they are the most stable form of these sugars under physiological conditions (**Figure 5.4**).

Monosaccharides, particularly glucose, are major nutrients for cells. In the process known as cellular respiration, cells extract energy from glucose molecules by breaking them down in a series of reactions. Not only are simple-sugar molecules a major fuel for cellular work, but their carbon skeletons also serve as raw material for the synthesis of other types of small organic molecules, such as amino acids and fatty acids. Sugar molecules that are not immediately used in these ways

are generally incorporated as monomers into disaccharides or polysaccharides, discussed next.

A **disaccharide** consists of two monosaccharides joined by a **glycosidic linkage**, a covalent bond formed between two monosaccharides by a dehydration reaction (*glyco* refers to carbohydrate). For example, maltose is a disaccharide formed by the linking of two molecules of glucose (**Figure 5.5a**). Also known as malt sugar, maltose is an ingredient used in brewing beer. The most prevalent disaccharide is sucrose, or table sugar. Its two monomers are glucose and fructose (**Figure 5.5b**). Plants generally transport carbohydrates from leaves to roots

▼ **Figure 5.5** Examples of disaccharide synthesis.



DRAW IT ► Referring to Figures 5.3 and 5.4, number the carbons in each sugar in this figure. How does the name of each linkage relate to the numbers?



Animation: Synthesis of Sucrose

and other nonphotosynthetic organs in the form of sucrose. Lactose, the sugar present in milk, is another disaccharide, in this case a glucose molecule joined to a galactose molecule. Disaccharides must be broken down into monosaccharides to be used for energy by organisms. Lactose intolerance is a common condition in humans who lack lactase, the enzyme that breaks down lactose. The sugar is instead broken down by intestinal bacteria, causing formation of gas and subsequent cramping. The problem may be avoided by taking the enzyme lactase when eating or drinking dairy products or consuming dairy products that have already been treated with lactase to break down the lactose.

Polysaccharides

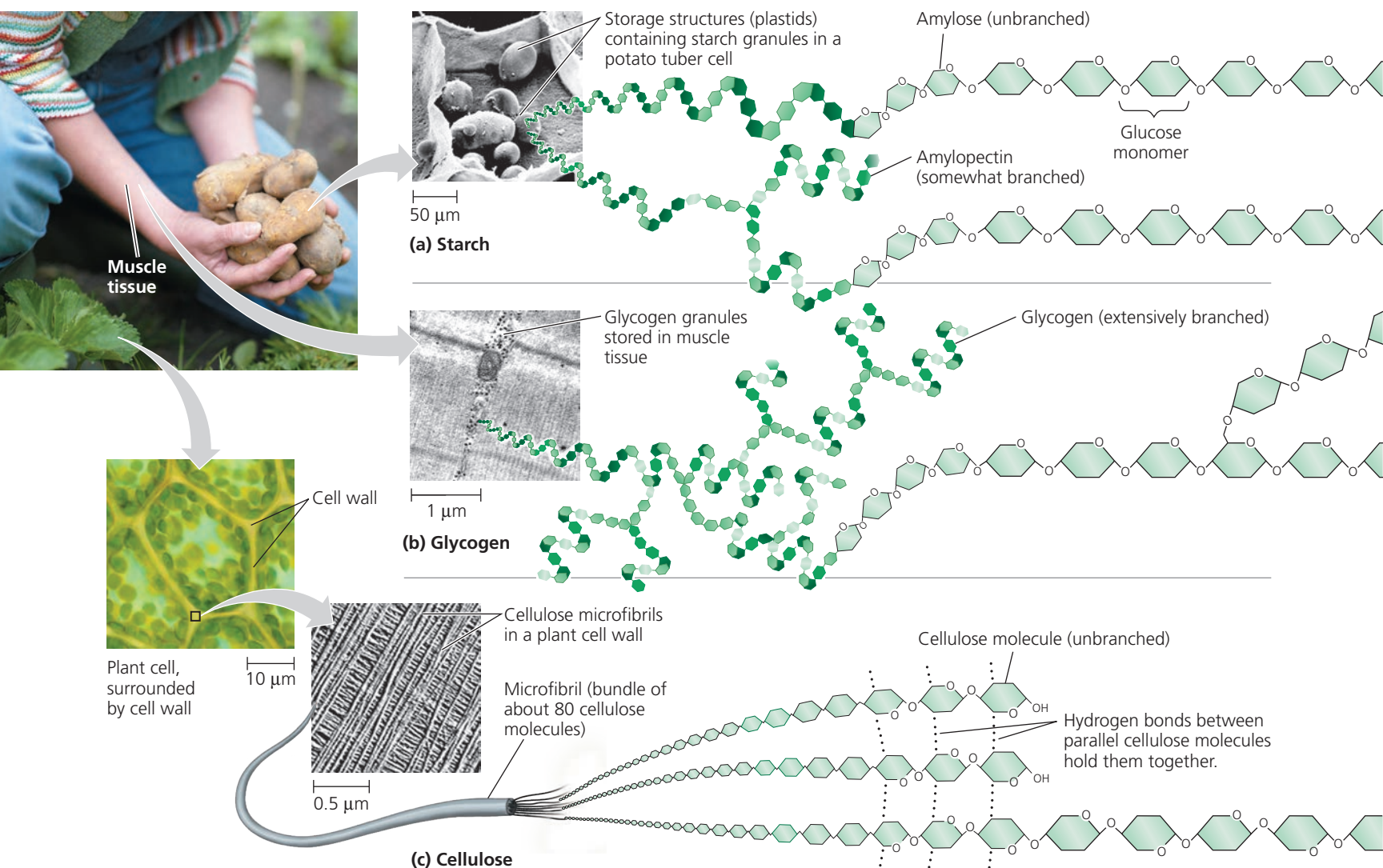
Polysaccharides are macromolecules, polymers with a few hundred to a few thousand monosaccharides joined by

glycosidic linkages. Some polysaccharides serve as storage material, hydrolyzed as needed to provide sugar for cells. Other polysaccharides serve as building material for structures that protect the cell or the whole organism. The architecture and function of a polysaccharide are determined by its sugar monomers and by the positions of its glycosidic linkages.

Storage Polysaccharides

Both plants and animals store sugars for later use in the form of storage polysaccharides (**Figure 5.6**). Plants store **starch**, a polymer of glucose monomers, as granules within cellular structures known as plastids. (Plastids include chloroplasts.) Synthesizing starch enables the plant to stockpile surplus glucose. Because glucose is a major cellular fuel, starch represents stored energy. The sugar can later be withdrawn by the plant from this carbohydrate “bank” by hydrolysis, which breaks the

▼ **Figure 5.6 Polysaccharides of plants and animals.** (a) Starch stored in plant cells, (b) glycogen stored in muscle cells, and (c) structural cellulose fibers in plant cell walls are all polysaccharides composed entirely of glucose monomers (green hexagons). In starch and glycogen, the polymer chains tend to form helices in unbranched regions because of the angle of the linkages between glucose molecules. There are two kinds of starch: amylose and amylopectin. Cellulose, with a different kind of glucose linkage, is always unbranched.



bonds between the glucose monomers. Most animals, including humans, also have enzymes that can hydrolyze plant starch, making glucose available as a nutrient for cells. Potato tubers and grains—the fruits of wheat, maize (corn), rice, and other grasses—are the major sources of starch in the human diet.

Most of the glucose monomers in starch are joined by 1–4 linkages (number 1 carbon to number 4 carbon), like the glucose units in maltose (see Figure 5.5a). The simplest form of starch, amylose, is unbranched. Amylopectin, a more complex starch, is a branched polymer with 1–6 linkages at the branch points. Both of these starches are shown in Figure 5.6a.

Animals store a polysaccharide called **glycogen**, a polymer of glucose that is like amylopectin but more extensively branched (Figure 5.6b). Vertebrates store glycogen mainly in liver and muscle cells. Hydrolysis of glycogen in these cells releases glucose when the demand for sugar increases. (The extensively branched structure of glycogen fits its function: More free ends are available for hydrolysis.) This stored fuel cannot sustain an animal for long, however. In humans, for example, glycogen stores are depleted in about a day unless they are replenished by eating. This is an issue of concern in low-carbohydrate diets, which can result in weakness and fatigue.

Structural Polysaccharides

Organisms build strong materials from structural polysaccharides. For example, the polysaccharide called **cellulose** is a major component of the tough walls that enclose plant cells (Figure 5.6c). Globally, plants produce almost 10^{14} kg (100 billion tons) of cellulose per year; it is the most abundant organic compound on Earth.

Like starch, cellulose is a polymer of glucose with 1–4 glycosidic linkages, but the linkages in these two polymers differ.

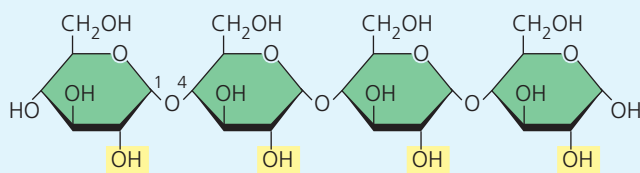
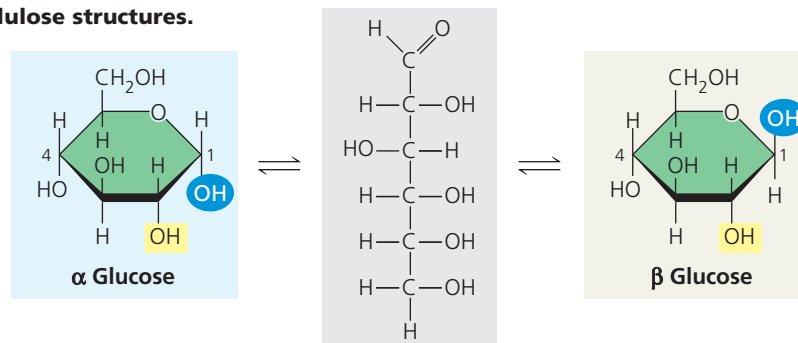
The difference is based on the fact that there are actually two slightly different ring structures for glucose (Figure 5.7a). When glucose forms a ring, the hydroxyl group attached to the number 1 carbon is positioned either below or above the plane of the ring. These two ring forms for glucose are called alpha (α) and beta (β), respectively. (Greek letters are often used as a “numbering” system for different versions of biological structures, much as we use the letters a, b, c, and so on for the parts of a question or a figure.) In starch, all the glucose monomers are in the α configuration (Figure 5.7b), the arrangement we saw in Figures 5.4 and 5.5. In contrast, the glucose monomers of cellulose are all in the β configuration, making every glucose monomer “upside down” with respect to its neighbors (Figure 5.7c; see also Figure 5.6c).

The differing glycosidic linkages in starch and cellulose give the two molecules distinct three-dimensional shapes. Certain starch molecules are largely helical, fitting their function of efficiently storing glucose units. Conversely, a cellulose molecule is straight. Cellulose is never branched, and some hydroxyl groups on its glucose monomers are free to hydrogen-bond with the hydroxyls of other cellulose molecules lying parallel to it. In plant cell walls, parallel cellulose molecules held together in this way are grouped into units called microfibrils (see Figure 5.6c). These cable-like microfibrils are a strong building material for plants and an important substance for humans because cellulose is the major constituent of paper and the only component of cotton. The unbranched structure of cellulose thus fits its function: imparting strength to parts of the plant.

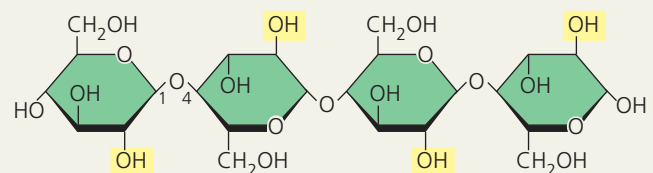
Enzymes that digest starch by hydrolyzing its α linkages are unable to hydrolyze the β linkages of cellulose due to the different shapes of these two molecules. In fact,

▼ **Figure 5.7 Starch and cellulose structures.**

(a) α and β glucose ring structures. These two interconvertible forms of glucose differ in the placement of the hydroxyl group (highlighted in blue) attached to the number 1 carbon.

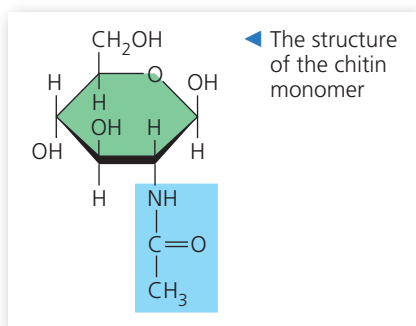


(b) Starch: 1–4 linkage of α glucose monomers. All monomers are in the same orientation. Compare the positions of the —OH groups highlighted in yellow with those in cellulose (c).



(c) Cellulose: 1–4 linkage of β glucose monomers. In cellulose, every β glucose monomer is upside down with respect to its neighbors. (See the highlighted —OH groups.)

▼ **Figure 5.8 Chitin, a structural polysaccharide.**



◀ Chitin, embedded in proteins, forms the exoskeleton of arthropods. This emperor dragonfly (*Anax imperator*) is molting—shedding its old exoskeleton (brown) and emerging upside down in adult form.

few organisms possess enzymes that can digest cellulose. Almost all animals, including humans, do not; the cellulose in our food passes through the digestive tract and is eliminated with the feces. Along the way, the cellulose abrades the wall of the digestive tract and stimulates the lining to secrete mucus, which aids in the smooth passage of food through the tract. Thus, although cellulose is not a nutrient for humans, it is an important part of a healthful diet. Most fruits, vegetables, and whole grains are rich in cellulose. On food packages, “insoluble fiber” refers mainly to cellulose.

Some microorganisms can digest cellulose, breaking it down into glucose monomers. A cow harbors cellulose-digesting prokaryotes and protists in its gut. These microbes hydrolyze the cellulose of hay and grass and convert the glucose to other compounds that nourish the cow. Similarly, a termite, which is unable to digest cellulose by itself, has prokaryotes or protists living in its gut that can make a meal of wood. Some fungi can also digest cellulose in soil and elsewhere, thereby helping recycle chemical elements within Earth’s ecosystems.

Another important structural polysaccharide is **chitin**, the carbohydrate used by arthropods (insects, spiders, crustaceans, and related animals) to build their exoskeletons (**Figure 5.8**). An exoskeleton is a hard case that surrounds the soft parts of an animal. Made up of chitin embedded in a layer of proteins, the case is leathery and flexible at first, but becomes hardened when the proteins are chemically linked to each other (as in insects) or encrusted with calcium carbonate (as in crabs). Chitin is also found in fungi, which use this polysaccharide rather than cellulose as the building material for their cell walls. Chitin is similar to cellulose, with β linkages, except that the glucose monomer of chitin has a nitrogen-containing attachment (see **Figure 5.8**).

CONCEPT CHECK 5.2

1. Write the formula for a monosaccharide that has three carbons.
2. A dehydration reaction joins two glucose molecules to form maltose. The formula for glucose is $C_6H_{12}O_6$. What is the formula for maltose?
3. **WHAT IF? >** After a cow is given antibiotics to treat an infection, a vet gives the animal a drink of “gut culture” containing various prokaryotes. Why is this necessary?

For suggested answers, see Appendix A.

CONCEPT 5.3

Lipids are a diverse group of hydrophobic molecules

Lipids are the one class of large biological molecules that does not include true polymers, and they are generally not big enough to be considered macromolecules. The compounds called **lipids** are grouped with each other because they share one important trait: They mix poorly, if at all, with water. The hydrophobic behavior of lipids is based on their molecular structure. Although they may have some polar bonds associated with oxygen, lipids consist mostly of hydrocarbon regions. Lipids are varied in form and function. They include waxes and certain pigments, but we will focus on the types of lipids that are most important biologically: fats, phospholipids, and steroids.

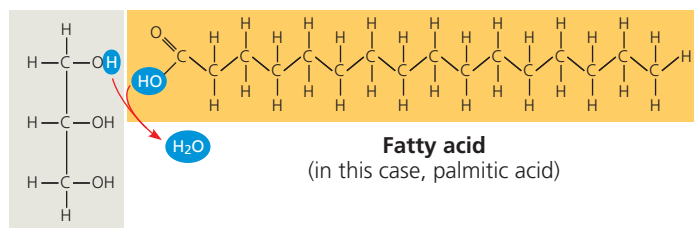
 **Animation: Lipids**

Fats

Although fats are not polymers, they are large molecules assembled from smaller molecules by dehydration reactions, like the dehydration reaction described for the polymerization of monomers in **Figure 5.2a**. A **fat** is constructed from two kinds of smaller molecules: glycerol and fatty acids (**Figure 5.9a**). Glycerol is an alcohol; each of its three carbons bears a hydroxyl group. A **fatty acid** has a long carbon skeleton, usually 16 or 18 carbon atoms in length. The carbon at one end of the skeleton is part of a carboxyl group, the functional group that gives these molecules the name fatty *acid*. The rest of the skeleton consists of a hydrocarbon chain. The relatively nonpolar C—H bonds in the hydrocarbon chains of fatty acids are the reason fats are hydrophobic. Fats separate from water because the water molecules hydrogen-bond to one another and exclude the fats. This is why vegetable oil (a liquid fat) separates from the aqueous vinegar solution in a bottle of salad dressing.

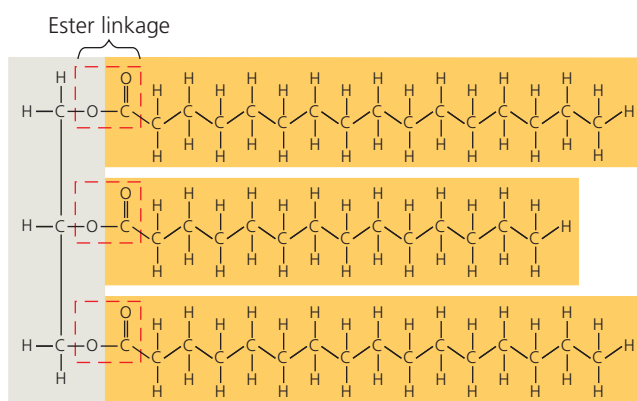
In making a fat, three fatty acid molecules are each joined to glycerol by an ester linkage, a bond formed by a dehydration reaction between a hydroxyl group and a carboxyl group. The resulting fat, also called a **triacylglycerol**, thus consists of three fatty acids linked to one glycerol molecule. (Still another name for a fat is *triglyceride*, a word often found

▼ **Figure 5.9 The synthesis and structure of a fat, or triacylglycerol.** The molecular building blocks of a fat are one molecule of glycerol and three molecules of fatty acids. **(a)** One water molecule is removed for each fatty acid joined to the glycerol. **(b)** A fat molecule with three fatty acid units, two of them identical. The carbons of the fatty acids are arranged zigzag to suggest the actual orientations of the four single bonds extending from each carbon (see Figures 4.3a and 4.6b).



Glycerol

(a) One of three dehydration reactions in the synthesis of a fat



(b) Fat molecule (triacylglycerol)

in the list of ingredients on packaged foods.) The fatty acids in a fat can all be the same, or they can be of two or three different kinds, as in **Figure 5.9b**.

The terms *saturated* fats and *unsaturated* fats are commonly used in the context of nutrition (**Figure 5.10**). These terms refer to the structure of the hydrocarbon chains of the fatty acids. If there are no double bonds between carbon atoms composing a chain, then as many hydrogen atoms as possible are bonded to the carbon skeleton. Such a structure is said to be *saturated* with hydrogen, and the resulting fatty acid is therefore called a **saturated fatty acid** (**Figure 5.10a**). An **unsaturated fatty acid** has one or more double bonds, with one fewer hydrogen atom on each double-bonded carbon. Nearly every double bond in naturally occurring fatty acids is a *cis* double bond, which creates a kink in the hydrocarbon chain wherever it occurs (**Figure 5.10b**). (See Figure 4.7b to remind yourself about *cis* and *trans* double bonds.)

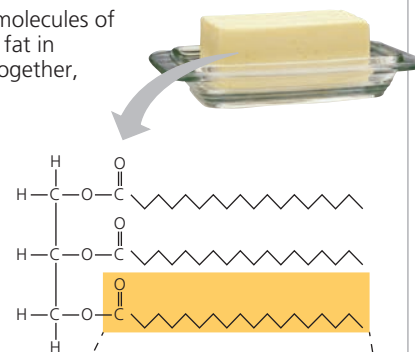
A fat made from saturated fatty acids is called a saturated fat. Most animal fats are saturated: The hydrocarbon chains of their fatty acids—the “tails” of the fat molecules—lack double bonds, and their flexibility allows the fat molecules to pack together tightly. Saturated animal fats—such as lard

▼ **Figure 5.10 Saturated and unsaturated fats and fatty acids.**

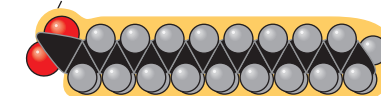
(a) Saturated fat

At room temperature, the molecules of a saturated fat, such as the fat in butter, are packed closely together, forming a solid.

Structural formula of a saturated fat molecule (Each hydrocarbon chain is represented as a zigzag line, where each bend represents a carbon atom; hydrogens are not shown.)



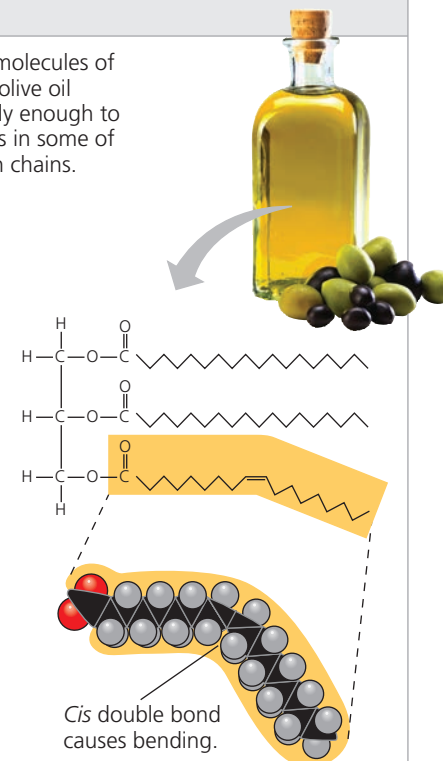
Space-filling model of stearic acid, a saturated fatty acid (red = oxygen, black = carbon, gray = hydrogen)



(b) Unsaturated fat

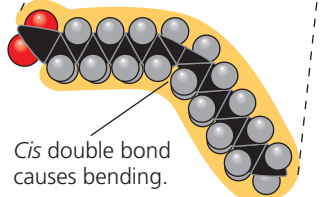
At room temperature, the molecules of an unsaturated fat such as olive oil cannot pack together closely enough to solidify because of the kinks in some of their fatty acid hydrocarbon chains.

Structural formula of an unsaturated fat molecule



Space-filling model of oleic acid, an unsaturated fatty acid

Cis double bond causes bending.



and butter—are solid at room temperature. In contrast, the fats of plants and fishes are generally unsaturated, meaning that they are built of one or more types of unsaturated fatty acids. Usually liquid at room temperature, plant and fish fats are referred to as oils—olive oil and cod liver oil are examples. The kinks where the *cis* double bonds are located prevent the molecules from packing together closely enough to solidify at room temperature. The phrase “hydrogenated vegetable oils”

on food labels means that unsaturated fats have been synthetically converted to saturated fats by adding hydrogen, allowing them to solidify. Peanut butter, margarine, and many other products are hydrogenated to prevent lipids from separating out in liquid (oil) form.

A diet rich in saturated fats is one of several factors that may contribute to the cardiovascular disease known as atherosclerosis. In this condition, deposits called plaques develop within the walls of blood vessels, causing inward bulges that impede blood flow and reduce the resilience of the vessels. The process of hydrogenating vegetable oils produces not only saturated fats but also unsaturated fats with *trans* double bonds. It appears that **trans fats** can contribute to coronary heart disease (see Concept 43.4). Because *trans* fats are especially common in baked goods and processed foods, the U.S. Food and Drug Administration (FDA) requires nutritional labels to include information on *trans* fat content. In addition, the FDA has ordered *trans* fats to be removed from the U.S. food supply by 2018. Some countries, such as Denmark and Switzerland, have already banned *trans* fats in foods.

The major function of fats is energy storage. The hydrocarbon chains of fats are similar to gasoline molecules and just as rich in energy. A gram of fat stores more than twice as much energy as a gram of a polysaccharide, such as starch. Because plants are relatively immobile, they can function with bulky energy storage in the form of starch. (Vegetable oils are generally obtained from seeds, where more compact storage is an asset to the plant.) Animals, however, must carry their energy stores with them, so there is an advantage to having a more compact reservoir of fuel—fat. Humans

and other mammals stock their long-term food reserves in adipose cells (see Figure 4.6a), which swell and shrink as fat is deposited and withdrawn from storage. In addition to storing energy, adipose tissue also cushions such vital organs as the kidneys, and a layer of fat beneath the skin insulates the body. This subcutaneous layer is especially thick in whales, seals, and most other marine mammals, insulating their bodies in cold ocean water.

Phospholipids

Cells as we know them could not exist without another type of lipid—phospholipids. Phospholipids are essential for cells because they are major constituents of cell membranes. Their structure provides a classic example of how form fits function at the molecular level. As shown in **Figure 5.11**, a **phospholipid** is similar to a fat molecule but has only two fatty acids attached to glycerol rather than three. The third hydroxyl group of glycerol is joined to a phosphate group, which has a negative electrical charge in the cell. Typically, an additional small charged or polar molecule is also linked to the phosphate group. Choline is one such molecule (see Figure 5.11), but there are many others as well, allowing formation of a variety of phospholipids that differ from each other.

The two ends of phospholipids show different behaviors with respect to water. The hydrocarbon tails are hydrophobic and are excluded from water. However, the phosphate group and its attachments form a hydrophilic head that has an affinity for water. When phospholipids are added to water, they self-assemble into a double-layered sheet called a “bilayer”

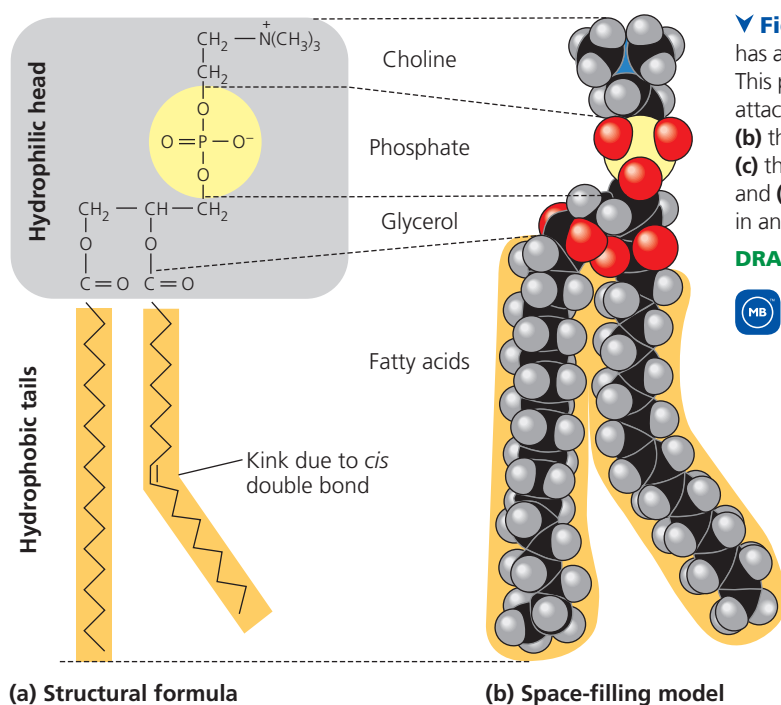
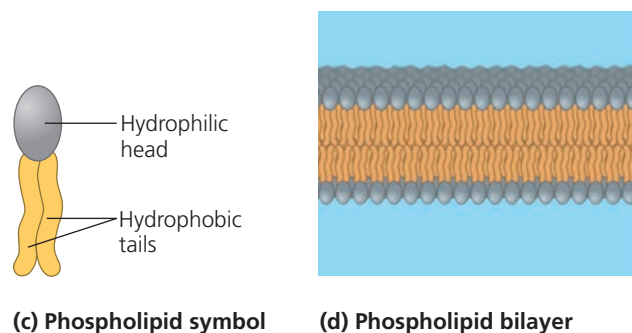


Figure 5.11 The structure of a phospholipid. A phospholipid has a hydrophilic (polar) head and two hydrophobic (nonpolar) tails. This particular phospholipid, called a phosphatidylcholine, has a choline attached to a phosphate group. Shown here are (a) the structural formula, (b) the space-filling model (yellow = phosphorus, blue = nitrogen), (c) the symbol for a phospholipid that will appear throughout this book, and (d) the bilayer structure formed by self-assembly of phospholipids in an aqueous environment.

DRAW IT Draw an oval around the hydrophilic head of the space-filling model.

Figure Walkthrough
Animation: Space-Filling Model of a Phospholipid



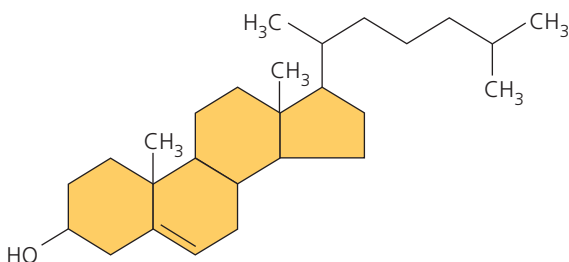
that shields their hydrophobic fatty acid tails from water (Figure 5.11d).

At the surface of a cell, phospholipids are arranged in a similar bilayer. The hydrophilic heads of the molecules are on the outside of the bilayer, in contact with the aqueous solutions inside and outside of the cell. The hydrophobic tails point toward the interior of the bilayer, away from the water. The phospholipid bilayer forms a boundary between the cell and its external environment and establishes separate compartments within eukaryotic cells; in fact, the existence of cells depends on the properties of phospholipids.

Steroids

Steroids are lipids characterized by a carbon skeleton consisting of four fused rings. Different steroids are distinguished by the particular chemical groups attached to this ensemble of rings. **Cholesterol**, a type of steroid, is a crucial molecule in animals (Figure 5.12). It is a common component of animal cell membranes and is also the precursor from which other steroids, such as the vertebrate sex hormones, are synthesized. In vertebrates, cholesterol is synthesized in the liver and is also obtained from the diet. A high level of cholesterol in the blood may contribute to atherosclerosis, although some researchers are questioning the roles of cholesterol and saturated fats in the development of this condition.

▼ **Figure 5.12 Cholesterol, a steroid.** Cholesterol is the molecule from which other steroids, including the sex hormones, are synthesized. Steroids vary in the chemical groups attached to their four interconnected rings (shown in gold).



MAKE CONNECTIONS ► Compare cholesterol with the sex hormones shown in the figure at the beginning of Concept 4.3. Circle the chemical groups that cholesterol has in common with estradiol; put a square around the chemical groups that cholesterol has in common with testosterone.



Interview with Lovell Jones: Investigating the effects of sex hormones on cancer (see the interview before Chapter 2)

CONCEPT CHECK 5.3

1. Compare the structure of a fat (triglyceride) with that of a phospholipid.
2. Why are human sex hormones considered lipids?
3. **WHAT IF?** ► Suppose a marine mammal is exposed to very low temperatures. Explain how it would maintain the fluidity of cell membranes.

For suggested answers, see Appendix A.

CONCEPT 5.4

Proteins include a diversity of structures, resulting in a wide range of functions

Nearly every dynamic function of a living being depends on proteins. In fact, the importance of proteins is underscored by their name, which comes from the Greek word *proteios*, meaning “first,” or “primary.” Proteins account for more than 50% of the dry mass of most cells, and they are instrumental in almost everything organisms do. Some proteins speed up chemical reactions, while others play a role in defense, storage, transport, cellular communication, movement, or structural support. **Figure 5.13** shows examples of proteins with these functions, which you’ll learn more about in later chapters.

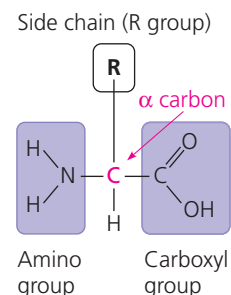
Life would not be possible without enzymes, most of which are proteins. Enzymatic proteins regulate metabolism by acting as **catalysts**, chemical agents that selectively speed up chemical reactions without being consumed in the reaction. Because an enzyme can perform its function over and over again, these molecules can be thought of as workhorses that keep cells running by carrying out the processes of life.

A human has tens of thousands of different proteins, each with a specific structure and function; proteins, in fact, are the most structurally sophisticated molecules known. Consistent with their diverse functions, they vary extensively in structure, each type of protein having a unique three-dimensional shape.

Proteins are all constructed from the same set of 20 amino acids, linked in unbranched polymers. The bond between amino acids is called a *peptide bond*, so a polymer of amino acids is called a **polypeptide**. A **protein** is a biologically functional molecule made up of one or more polypeptides, each folded and coiled into a specific three-dimensional structure.

Amino Acid Monomers

All amino acids share a common structure. An **amino acid** is an organic molecule with both an amino group and a carboxyl group (see Figure 4.9); the small figure shows the general formula for an amino acid. At the center of the amino acid is an asymmetric carbon atom called the *alpha* (α) *carbon*. Its four different partners are an amino group, a carboxyl group, a hydrogen atom, and a variable group symbolized by R. The R group, also called the side chain, differs with each amino acid.



✓ **Figure 5.13** An overview of protein functions.

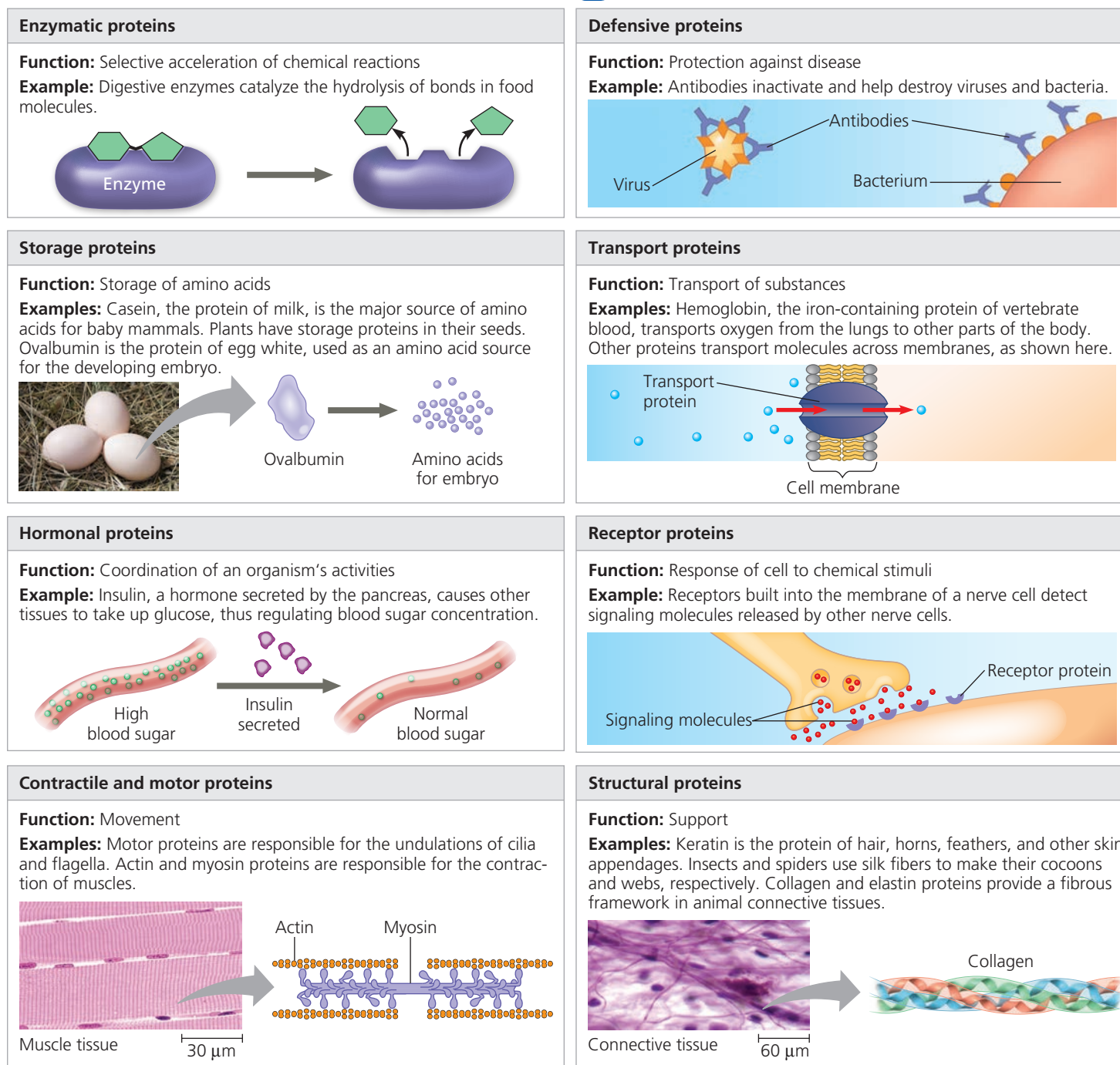
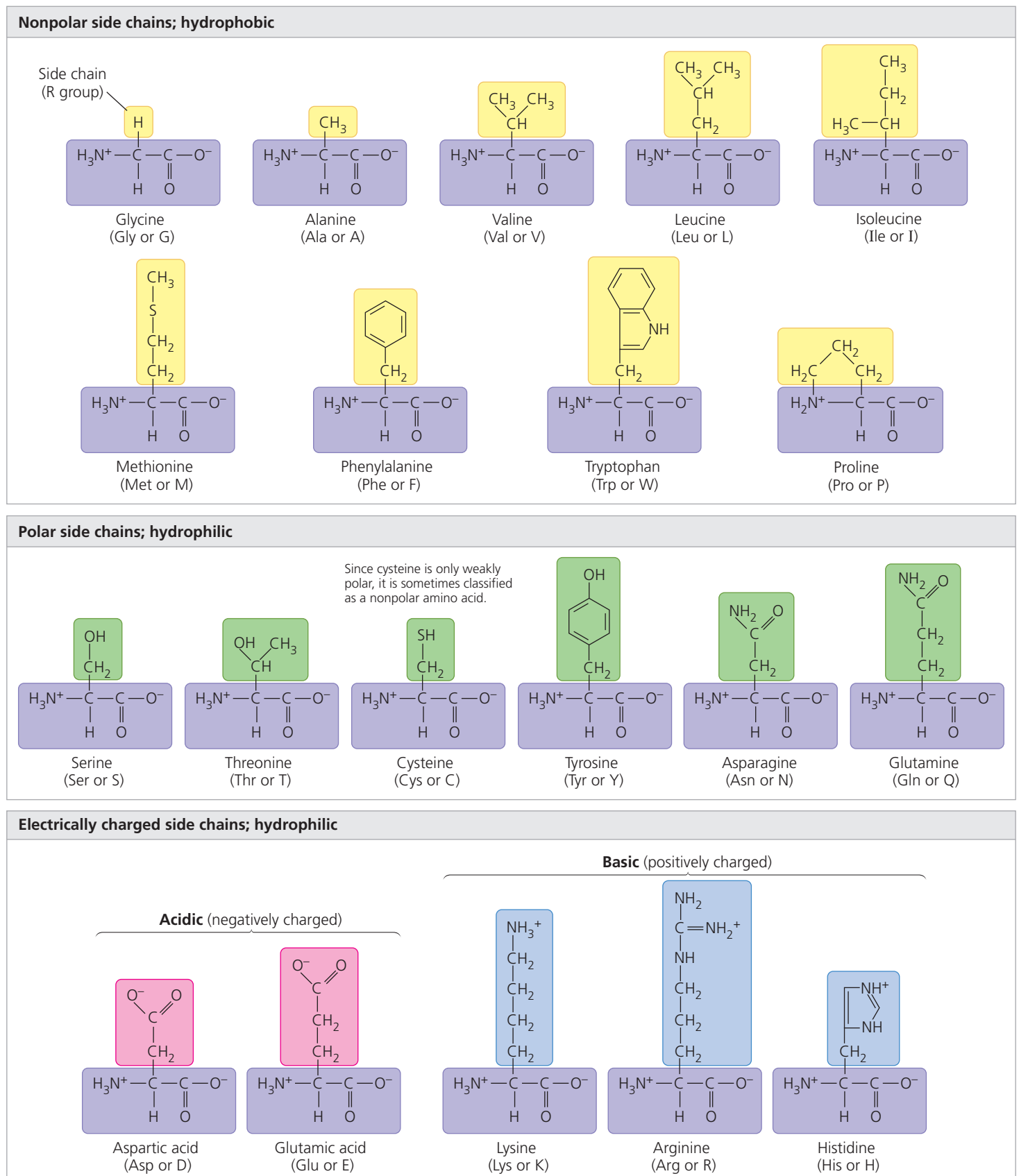


Figure 5.14 shows the 20 amino acids that cells use to build their thousands of proteins. Here the amino groups and carboxyl groups are all depicted in ionized form, the way they usually exist at the pH found in a cell. The side chain (R group) may be as simple as a hydrogen atom, as in the amino acid glycine, or it may be a carbon skeleton with various functional groups attached, as in glutamine.

The physical and chemical properties of the side chain determine the unique characteristics of a particular amino acid, thus affecting its functional role in a polypeptide. In Figure 5.14, the amino acids are grouped according to the properties of their

side chains. One group consists of amino acids with nonpolar side chains, which are hydrophobic. Another group consists of amino acids with polar side chains, which are hydrophilic. Acidic amino acids have side chains that are generally negative in charge due to the presence of a carboxyl group, which is usually dissociated (ionized) at cellular pH. Basic amino acids have amino groups in their side chains that are generally positive in charge. (Notice that *all* amino acids have carboxyl groups and amino groups; the terms *acidic* and *basic* in this context refer only to groups in the side chains.) Because they are charged, acidic and basic side chains are also hydrophilic.

▼ **Figure 5.14 The 20 amino acids of proteins.** The amino acids are grouped here according to the properties of their side chains (R groups) and shown in their prevailing ionic forms at pH 7.2, the pH within a cell. The three-letter and one-letter abbreviations for the amino acids are in parentheses. All of the amino acids used in proteins are L enantiomers (see Figure 4.7c).

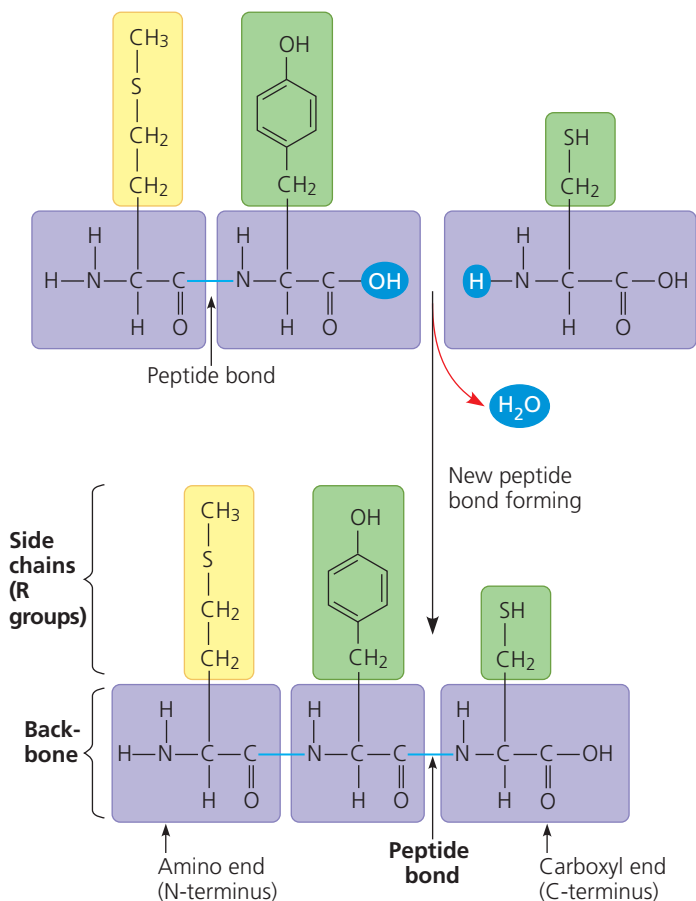


Polypeptides (Amino Acid Polymers)

Now that we have examined amino acids, let's see how they are linked to form polymers (**Figure 5.15**). When two amino acids are positioned so that the carboxyl group of one is adjacent to the amino group of the other, they can become joined by a dehydration reaction, with the removal of a water molecule. The resulting covalent bond is called a **peptide bond**. Repeated over and over, this process yields a polypeptide, a polymer of many amino acids linked by peptide bonds. You'll learn more about how cells synthesize polypeptides in Concept 17.4.

The repeating sequence of atoms highlighted in purple in **Figure 5.15** is called the *polypeptide backbone*. Extending from this backbone are the different side chains (R groups) of the amino acids. Polypeptides range in length from a few amino acids to 1,000 or more. Each specific polypeptide has a unique linear sequence of amino acids. Note that one end of the polypeptide chain has a free amino group (the N-terminus of the polypeptide), while the opposite end has a free carboxyl group (the C-terminus). The chemical nature of the molecule

▼ **Figure 5.15 Making a polypeptide chain.** Peptide bonds are formed by dehydration reactions, which link the carboxyl group of one amino acid to the amino group of the next. The peptide bonds are formed one at a time, starting with the amino acid at the amino end (N-terminus). The polypeptide has a repetitive backbone (purple) to which the amino acid side chains (yellow and green) are attached.



DRAW IT ► Label the three amino acids in the upper part of the figure using three-letter and one-letter codes. Circle and label the carboxyl and amino groups that will form the new peptide bond.

as a whole is determined by the kind and sequence of the side chains, which determine how a polypeptide folds and thus its final shape and chemical characteristics. The immense variety of polypeptides in nature illustrates an important concept introduced earlier—that cells can make many different polymers by linking a limited set of monomers into diverse sequences.

Protein Structure and Function

The specific activities of proteins result from their intricate three-dimensional architecture, the simplest level of which is the sequence of their amino acids. What can the amino acid sequence of a polypeptide tell us about the three-dimensional structure (commonly referred to simply as the “structure”) of the protein and its function? The term *polypeptide* is not synonymous with the term *protein*. Even for a protein consisting of a single polypeptide, the relationship is somewhat analogous to that between a long strand of yarn and a sweater of particular size and shape that can be knitted from the yarn. A functional protein is not *just* a polypeptide chain, but one or more polypeptides precisely twisted, folded, and coiled into a molecule of unique shape, which can be shown in several different types of models (**Figure 5.16**). And it is the amino acid sequence of each polypeptide that determines what three-dimensional structure the protein will have under normal cellular conditions.

When a cell synthesizes a polypeptide, the chain may fold spontaneously, assuming the functional structure for that protein. This folding is driven and reinforced by the formation of various bonds between parts of the chain, which in turn depends on the sequence of amino acids. Many proteins are roughly spherical (*globular proteins*), while others are shaped like long fibers (*fibrous proteins*). Even within these broad categories, countless variations exist.

A protein's specific structure determines how it works. In almost every case, the function of a protein depends on its ability to recognize and bind to some other molecule. In an especially striking example of the marriage of form and function, **Figure 5.17** shows the exact match of shape between an antibody (a protein in the body) and the particular foreign substance on a flu virus that the antibody binds to and marks for destruction. Also, you may recall another example of molecules with matching shapes from Concept 2.3: endorphin molecules (produced by the body) and morphine molecules (a manufactured drug), both of which fit into receptor proteins on the surface of brain cells in humans, producing euphoria and relieving pain. Morphine, heroin, and other opiate drugs are able to mimic endorphins because they all have a shape similar to that of endorphins and can thus fit into and bind to endorphin receptors in the brain. This fit is very specific, something like a lock and key (see **Figure 2.16**). The endorphin receptor, like other receptor molecules, is a protein. The function of a protein—for instance, the ability of a receptor protein to bind to a particular pain-relieving signaling molecule—is an emergent property resulting from exquisite molecular order.

▼ Figure 5.16 Visualizing Proteins

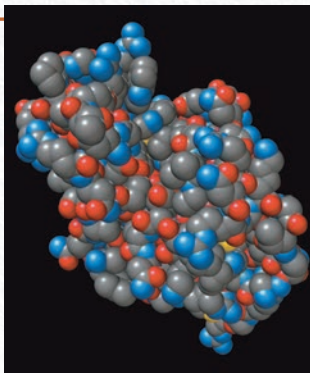
Proteins can be represented in different ways, depending on the goal of the illustration.

Structural Models

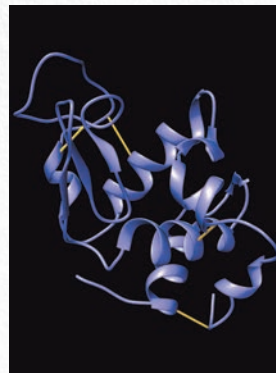
Using data from structural studies of proteins, computers can generate various types of models. Each model emphasizes a different aspect of the protein's structure, but no model can show what a protein actually looks like. These three models depict lysozyme, a protein in tears and saliva that helps prevent infection by binding to target molecules on bacteria.

1 In which model is it easiest to follow the polypeptide backbone?

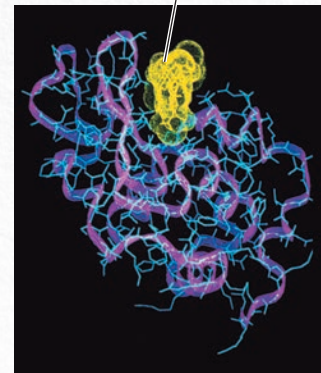
Instructors: The tutorial "Molecular Model: Lysozyme," in which students rotate 3-D models of lysozyme, can be assigned in MasteringBiology.



Space-filling model: Shows all the atoms of the protein (except hydrogen), emphasizing the overall globular shape. The atoms are color-coded: gray = carbon, red = oxygen, blue = nitrogen, and yellow = sulfur.



Ribbon model: Shows only the backbone of the polypeptide, emphasizing how it folds and coils to form a 3-D shape, in this case stabilized by disulfide bridges (yellow lines).

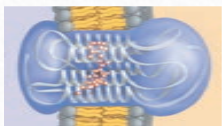


Wireframe model (blue): Shows the backbone of the polypeptide chain with side chains (R groups) extending from it (see Figure 5.15). A ribbon model (purple) is superimposed on the wireframe model.

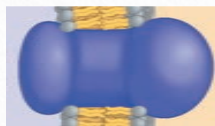
Target molecule (on bacterial cell surface) bound to lysozyme

Simplified Diagrams

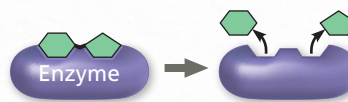
It isn't always necessary to use a detailed computer model; simplified diagrams are useful when the focus of the figure is on the function of the protein, not the structure.



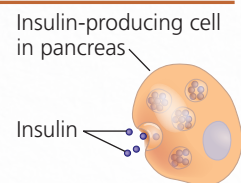
In this diagram of the protein rhodopsin, a simple transparent shape is drawn around the contours of a ribbon model, showing the overall shape of the molecule as well as some internal details.



When structural details are not needed, a solid shape can be used to represent a protein.



A simple shape is used here to represent a generic enzyme because the diagram focuses on enzyme action in general.



Sometimes a protein is represented simply as a dot, as shown here for insulin.

2 Draw a simple version of lysozyme that shows its overall shape, based on the molecular models in the top section of the figure.

3 Why is it unnecessary to show the actual shape of insulin here?

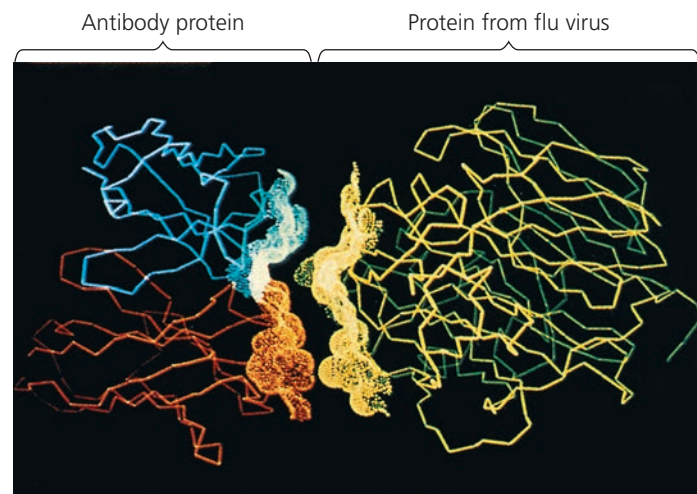
► **Figure 5.17 Complementarity of shape between two protein surfaces.** A technique called X-ray crystallography was used to generate a computer model of an antibody protein (blue and orange, left) bound to a flu virus protein (yellow and green, right). This is a wireframe model modified by adding an "electron density map" in the region where the two proteins meet. Computer software was then used to back the images away from each other slightly.

Four Levels of Protein Structure

In spite of their great diversity, proteins share three superimposed levels of structure, known as primary, secondary, and tertiary structure. A fourth level, quaternary structure, arises when a protein consists of two or more polypeptide chains.

Figure 5.18 describes these four levels of protein structure. Be sure to study this figure thoroughly before going on to the next section.

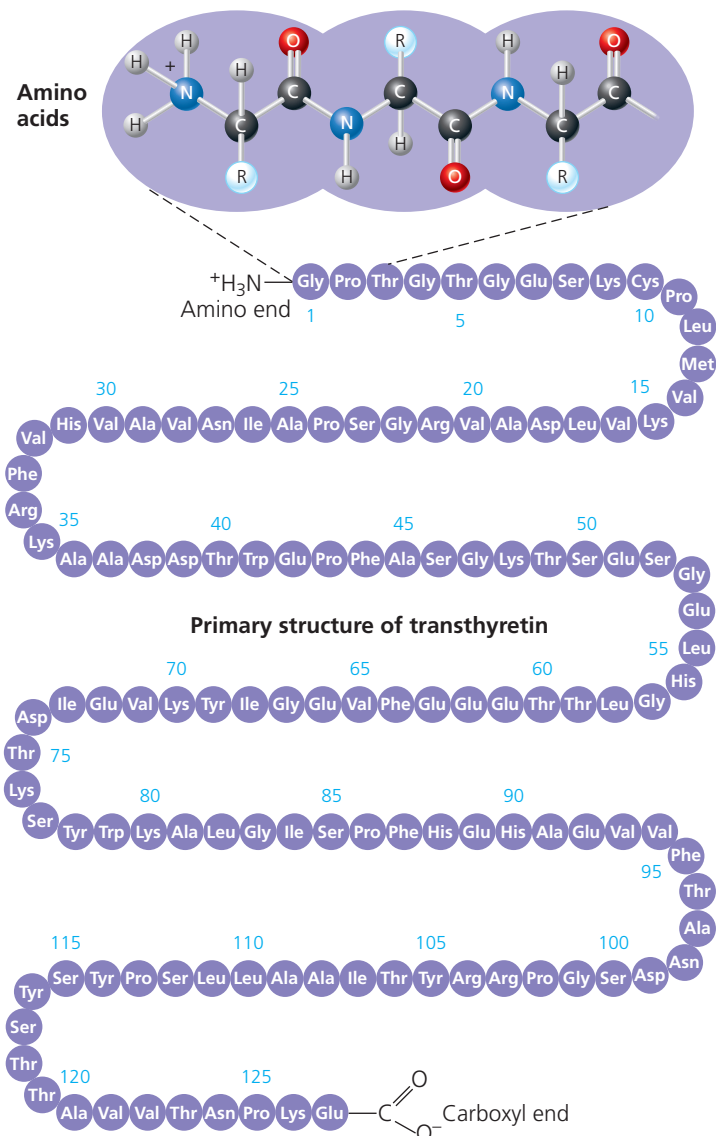
Animation: Protein Structure



VISUAL SKILLS ► What do these computer models allow you to see about the two proteins?

Primary Structure

Linear chain of amino acids

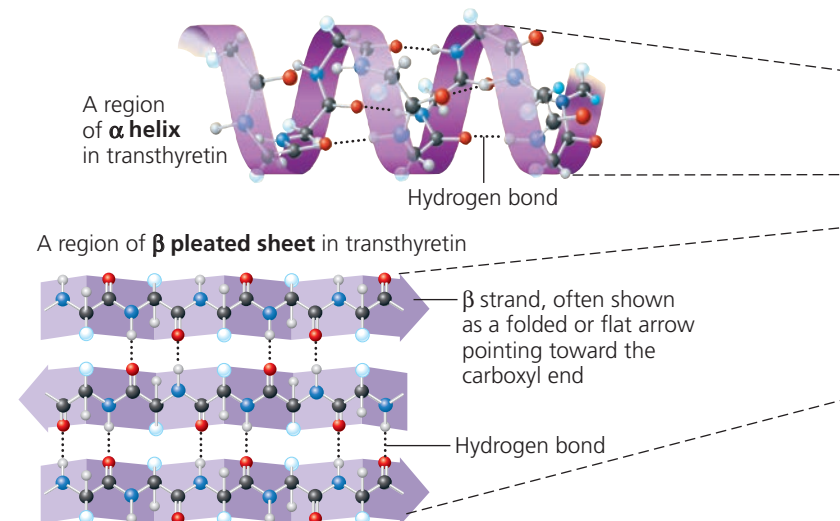


The **primary structure** of a protein is its sequence of amino acids. As an example, let's consider transthyretin, a globular blood protein that transports vitamin A and one of the thyroid hormones throughout the body. Transthyretin is made up of four identical polypeptide chains, each composed of 127 amino acids. Shown here is one of these chains unraveled for a closer look at its primary structure. Each of the 127 positions along the chain is occupied by one of the 20 amino acids, indicated here by its three-letter abbreviation.

The primary structure is like the order of letters in a very long word. If left to chance, there would be 20^{127} different ways of making a polypeptide chain 127 amino acids long. However, the precise primary structure of a protein is determined not by the random linking of amino acids, but by inherited genetic information. The primary structure in turn dictates secondary and tertiary structure, due to the chemical nature of the backbone and the side chains (R groups) of the amino acids along the polypeptide.

Secondary Structure

Regions stabilized by hydrogen bonds between atoms of the polypeptide backbone



Most proteins have segments of their polypeptide chains repeatedly coiled or folded in patterns that contribute to the protein's overall shape. These coils and folds, collectively referred to as **secondary structure**, are the result of hydrogen bonds between the repeating constituents of the polypeptide backbone (not the amino acid side chains). Within the backbone, the oxygen atoms have a partial negative charge, and the hydrogen atoms attached to the nitrogens have a partial positive charge (see Figure 2.14); therefore, hydrogen bonds can form between these atoms. Individually, these hydrogen bonds are weak, but because they are repeated many times over a relatively long region of the polypeptide chain, they can support a particular shape for that part of the protein.

One such secondary structure is the **α helix**, a delicate coil held together by hydrogen bonding between every fourth amino acid, as shown above. Although each transthyretin polypeptide has only one α helix region (see the Tertiary Structure section), other globular proteins have multiple stretches of α helix separated by nonhelical regions (see hemoglobin in the Quaternary Structure section). Some fibrous proteins, such as α -keratin, the structural protein of hair, have the α helix formation over most of their length.

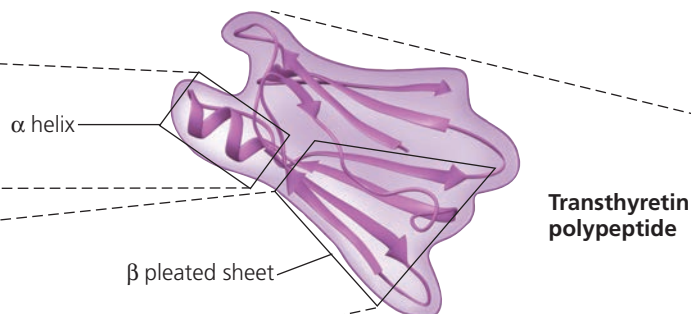
The other main type of secondary structure is the **β pleated sheet**. As shown above, in this structure two or more segments of the polypeptide chain lying side by side (called β strands) are connected by hydrogen bonds between parts of the two parallel segments of polypeptide backbone. β pleated sheets make up the core of many globular proteins, as is the case for transthyretin (see Tertiary Structure), and dominate some fibrous proteins, including the silk protein of a spider's web. The teamwork of so many hydrogen bonds makes each spider silk fiber stronger than a steel strand of the same weight.

► Spiders secrete silk fibers made of a structural protein containing β pleated sheets, which allow the spider web to stretch and recoil.



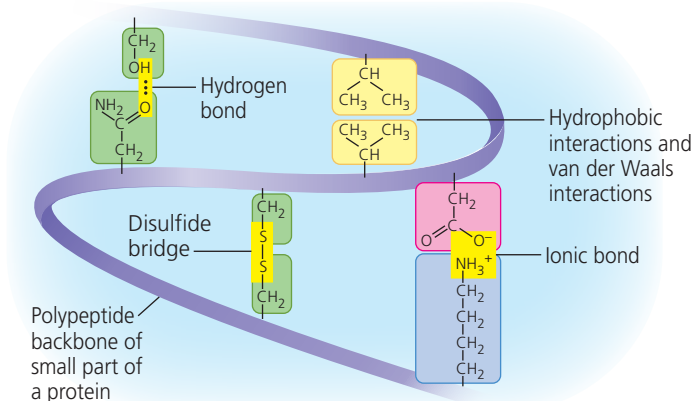
Tertiary Structure

Three-dimensional shape stabilized by interactions between side chains



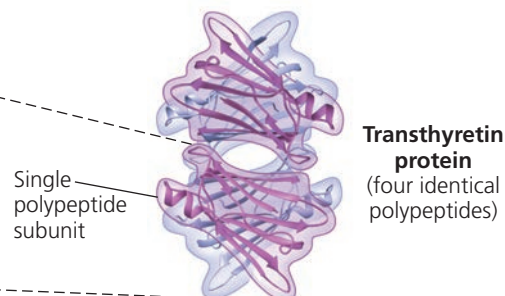
Superimposed on the patterns of secondary structure is a protein's tertiary structure, shown here in a ribbon model of the transthyretin polypeptide. While secondary structure involves interactions between backbone constituents, **tertiary structure** is the overall shape of a polypeptide resulting from interactions between the side chains (R groups) of the various amino acids. One type of interaction that contributes to tertiary structure is called—somewhat misleadingly—a **hydrophobic interaction**. As a polypeptide folds into its functional shape, amino acids with hydrophobic (nonpolar) side chains usually end up in clusters at the core of the protein, out of contact with water. Thus, a “hydrophobic interaction” is actually caused by the exclusion of nonpolar substances by water molecules. Once nonpolar amino acid side chains are close together, van der Waals interactions help hold them together. Meanwhile, hydrogen bonds between polar side chains and ionic bonds between positively and negatively charged side chains also help stabilize tertiary structure. These are all weak interactions in the aqueous cellular environment, but their cumulative effect helps give the protein a unique shape.

Covalent bonds called **disulfide bridges** may further reinforce the shape of a protein. Disulfide bridges form where two cysteine monomers, which have sulfhydryl groups ($-\text{SH}$) on their side chains (see Figure 4.9), are brought close together by the folding of the protein. The sulfur of one cysteine bonds to the sulfur of the second, and the disulfide bridge ($-\text{S}-\text{S}-$) rivets parts of the protein together (see yellow lines in Figure 5.16 ribbon model). All of these different kinds of interactions can contribute to the tertiary structure of a protein, as shown here in a small part of a hypothetical protein:



Quaternary Structure

Association of two or more polypeptides (some proteins only)

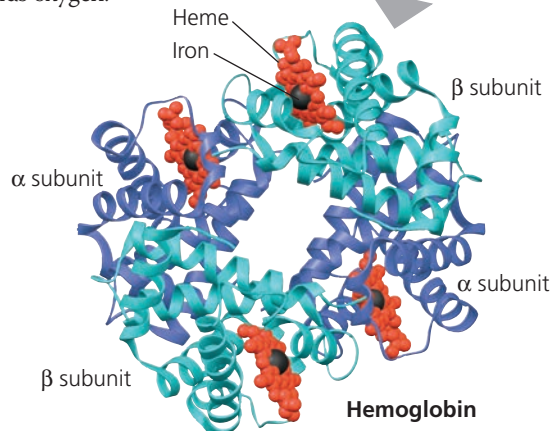


Some proteins consist of two or more polypeptide chains aggregated into one functional macromolecule. **Quaternary structure** is the overall protein structure that results from the aggregation of these polypeptide subunits. For example, shown above is the complete globular transthyretin protein, made up of its four polypeptides.

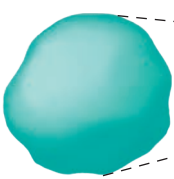
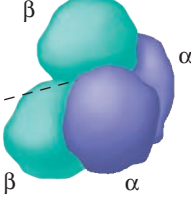
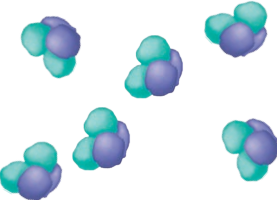
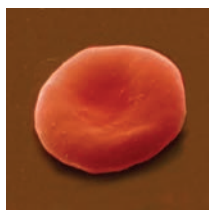
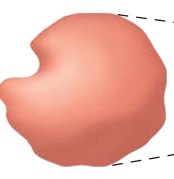
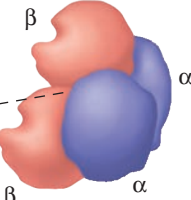
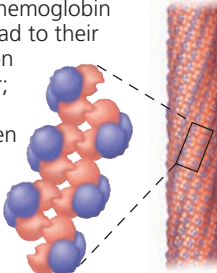
Another example is collagen, which is a fibrous protein that has three identical helical polypeptides intertwined into a larger triple helix, giving the long fibers great strength. This suits collagen fibers to their function as the girders of connective tissue in skin, bone, tendons, ligaments, and other body parts. (Collagen accounts for 40% of the protein in a human body.)



Hemoglobin, the oxygen-binding protein of red blood cells, is another example of a globular protein with quaternary structure. It consists of four polypeptide subunits, two of one kind (α) and two of another kind (β). Both α and β subunits consist primarily of α -helical secondary structure. Each subunit has a nonpolypeptide component, called heme, with an iron atom that binds oxygen.



▼ **Figure 5.19** A single amino acid substitution in a protein causes sickle-cell disease.

	Primary Structure	Secondary and Tertiary Structures	Quaternary Structure	Function	Red Blood Cell Shape
Normal hemoglobin	1 Val 2 His 3 Leu 4 Thr 5 Pro 6 Glu 7 Glu	Normal β subunit 	Normal hemoglobin 	Normal hemoglobin proteins do not associate with one another; each carries oxygen. 	Normal red blood cells are full of individual hemoglobin proteins.  5 μm
Sickle-cell hemoglobin	1 Val 2 His 3 Leu 4 Thr 5 Pro 6 Val 7 Glu	Sickle-cell β subunit 	Sickle-cell hemoglobin 	Hydrophobic interactions between sickle-cell hemoglobin proteins lead to their aggregation into a fiber; capacity to carry oxygen is greatly reduced. 	Fibers of abnormal hemoglobin deform red blood cell into sickle shape.  5 μm

MAKE CONNECTIONS ► Considering the chemical characteristics of the amino acids valine and glutamic acid (see Figure 5.14), propose a possible explanation for the dramatic effect on protein function that occurs when valine is substituted for glutamic acid.



HHMI Animation: Sickle-Cell Disease



Sickle-Cell Disease: A Change in Primary Structure

Even a slight change in primary structure can affect a protein's shape and ability to function. For instance, **sickle-cell disease**, an inherited blood disorder, is caused by the substitution of one amino acid (valine) for the normal one (glutamic acid) at the position of the sixth amino acid in the primary structure of hemoglobin, the protein that carries oxygen in red blood cells. Normal red blood cells are disk-shaped, but in sickle-cell disease, the abnormal hemoglobin molecules tend to aggregate into chains, deforming some of the cells into a sickle shape (Figure 5.19). A person with the disease has periodic "sickle-cell crises" when the angular cells clog tiny blood vessels, impeding blood flow. The toll taken on such patients is a dramatic example of how a simple change in protein structure can have devastating effects on protein function.



Interview with Linus Pauling: Winner of the Nobel Prize in Chemistry and the Nobel Peace Prize

What Determines Protein Structure?

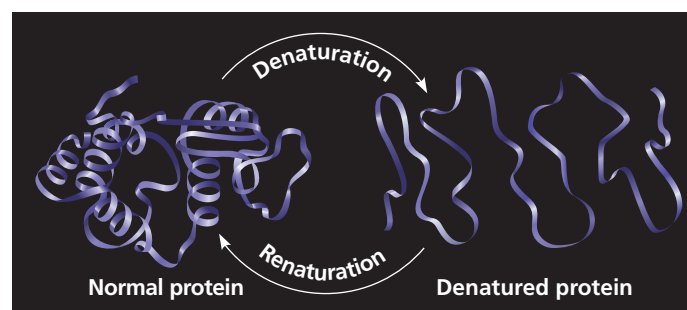
You've learned that a unique shape endows each protein with a specific function. But what are the key factors determining protein structure? You already know most of the answer: A polypeptide chain of a given amino acid sequence can be arranged into a three-dimensional shape determined by the interactions responsible for secondary and tertiary structure. This folding normally occurs as the protein is being

synthesized in the crowded environment within a cell, aided by other proteins. However, protein structure also depends on the physical and chemical conditions of the protein's environment. If the pH, salt concentration, temperature, or other aspects of its environment are altered, the weak chemical bonds and interactions within a protein may be destroyed, causing the protein to unravel and lose its native shape, a change called **denaturation** (Figure 5.20). Because it is misshapen, the denatured protein is biologically inactive.

Most proteins become denatured if they are transferred from an aqueous environment to a nonpolar solvent, such as ether or chloroform; the polypeptide chain refolds so that

▼ Figure 5.20 Denaturation and renaturation of a protein.

High temperatures or various chemical treatments will denature a protein, causing it to lose its shape and hence its ability to function. If the denatured protein remains dissolved, it may renature when the chemical and physical aspects of its environment are restored to normal.



its hydrophobic regions face outward toward the solvent. Other denaturation agents include chemicals that disrupt the hydrogen bonds, ionic bonds, and disulfide bridges that maintain a protein's shape. Denaturation can also result from excessive heat, which agitates the polypeptide chain enough to overpower the weak interactions that stabilize the structure. The white of an egg becomes opaque during cooking because the denatured proteins are insoluble and solidify. This also explains why excessively high fevers can be fatal: Proteins in the blood tend to denature at very high body temperatures.

When a protein in a test-tube solution has been denatured by heat or chemicals, it can sometimes return to its functional shape when the denaturing agent is removed. (Sometimes this is not possible: For example, a fried egg will not become liquefied when placed back into the refrigerator!) We can conclude that the information for building specific shape is intrinsic to the protein's primary structure; this is often the case for small proteins. The sequence of amino acids determines the protein's shape—where an α helix can form, where β pleated sheets can exist, where disulfide bridges are located, where ionic bonds can form, and so on. But how does protein folding occur in the cell?

Protein Folding in the Cell

Biochemists now know the amino acid sequence for about 65 million proteins, with roughly 1.5 million added each month, and the three-dimensional shape for almost 35,000. Researchers have tried to correlate the primary structure of many proteins with their three-dimensional structure to discover the rules of protein folding. Unfortunately, however, the protein-folding process is not that simple. Most proteins probably go through several intermediate structures on their way to a stable shape, and looking at the mature structure does not reveal the stages of folding required to achieve that form. However, biochemists have developed methods for tracking a protein through such stages and learning more about this important process.

Misfolding of polypeptides in cells is a serious problem that has come under increasing scrutiny by medical researchers. Many diseases—such as cystic fibrosis, Alzheimer's, Parkinson's, and mad cow disease—are associated with an accumulation of misfolded proteins. In fact, misfolded versions of the transthyretin protein featured in Figure 5.18 have been implicated in several diseases, including one form of senile dementia.

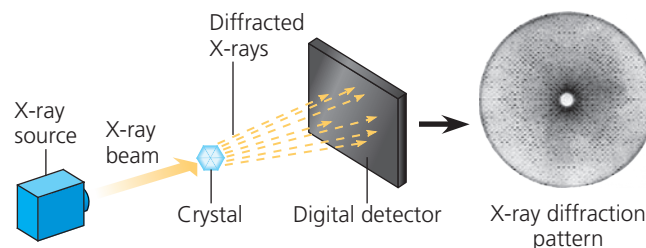
Even when scientists have a correctly folded protein in hand, determining its exact three-dimensional structure is not simple, for a single protein has thousands of atoms. The method most commonly used to determine the 3-D structure of a protein is **X-ray crystallography**, which depends on the diffraction of an X-ray beam by the atoms of a crystallized molecule. Using this technique, scientists can build a 3-D model that shows the exact position of every atom in a protein molecule (**Figure 5.21**). Nuclear magnetic resonance (NMR)

Figure 5.21

Research Method X-Ray Crystallography

Application Scientists use X-ray crystallography to determine the three-dimensional (3-D) structure of macromolecules such as nucleic acids and proteins.

Technique Researchers aim an X-ray beam through a crystallized protein or nucleic acid. The atoms of the crystal diffract (bend) the X-rays into an orderly array that a digital detector records as a pattern of spots called an X-ray diffraction pattern, an example of which is shown here.



Results Using data from X-ray diffraction patterns and the sequence of monomers determined by chemical methods, researchers can build a 3-D computer model of the macromolecule being studied, such as the four-subunit protein transthyretin (see Figure 5.18) shown here.



spectroscopy and bioinformatics (see Concept 5.6) are complementary approaches to understanding protein structure and function.

The structure of some proteins is difficult to determine for a simple reason: A growing body of biochemical research has revealed that a significant number of proteins, or regions of proteins, do not have a distinct 3-D structure until they interact with a target protein or other molecule. Their flexibility and indefinite structure are important for their function, which may require binding with different targets at different times. These proteins, which may account for 20–30% of mammalian proteins, are called *intrinsically disordered proteins* and are the focus of current research.

CONCEPT CHECK 5.4

1. What parts of a polypeptide participate in the bonds that hold together secondary structure? Tertiary structure?
2. Thus far in the chapter, the Greek letters α and β have been used to specify at least three different pairs of structures. Name and briefly describe them.
3. **WHAT IF?** > Where would you expect a polypeptide region rich in the amino acids valine, leucine, and isoleucine to be located in a folded polypeptide? Explain.

For suggested answers, see Appendix A.

CONCEPT 5.5

Nucleic acids store, transmit, and help express hereditary information

If the primary structure of polypeptides determines a protein's shape, what determines primary structure? The amino acid sequence of a polypeptide is programmed by a discrete unit of inheritance known as a **gene**. Genes consist of DNA, which belongs to the class of compounds called nucleic acids. **Nucleic acids** are polymers made of monomers called nucleotides.

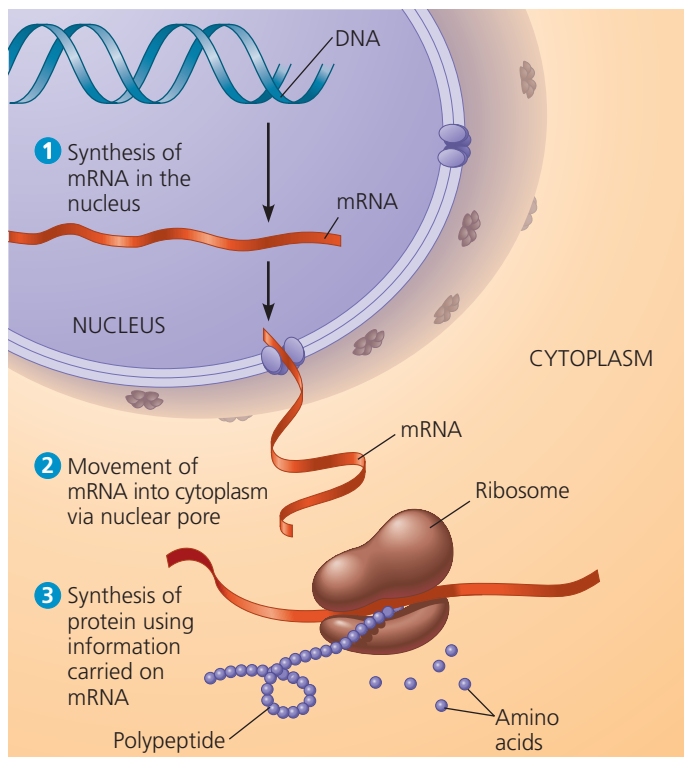
The Roles of Nucleic Acids

The two types of nucleic acids, **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**, enable living organisms to reproduce their complex components from one generation to the next. Unique among molecules, DNA provides directions for its own replication. DNA also directs RNA synthesis and, through RNA, controls protein synthesis; this entire process is called **gene expression (Figure 5.22)**.

DNA is the genetic material that organisms inherit from their parents. Each chromosome contains one long DNA molecule, usually carrying several hundred or more genes. When a cell reproduces itself by dividing, its DNA molecules are copied and passed along from one generation of cells to the next. The information that programs all the cell's activities is encoded in the structure of the DNA. The DNA, however, is not directly involved in running the operations of the cell, any more than computer software by itself can read the bar code on a box of cereal. Just as a scanner is needed to read a bar code, proteins are required to implement genetic programs. The molecular hardware of the cell—the tools that carry out biological functions—consists mostly of proteins. For example, the oxygen carrier in red blood cells is the protein hemoglobin that you saw earlier (see Figure 5.18), not the DNA that specifies its structure.

How does RNA, the other type of nucleic acid, fit into gene expression, the flow of genetic information from DNA to proteins? A given gene along a DNA molecule can direct synthesis of a type of RNA called *messenger RNA (mRNA)*. The mRNA molecule interacts with the cell's protein-synthesizing machinery to direct production of a polypeptide, which folds into all or part of a protein. We can summarize the flow of genetic information as DNA → RNA → protein (see Figure 5.22). The sites of protein synthesis are cellular structures called ribosomes. In a eukaryotic cell, ribosomes are in the cytoplasm—the region between the nucleus and the plasma membrane, the cell's outer boundary—but DNA resides in the nucleus. Messenger RNA conveys genetic instructions for building proteins from the nucleus to the cytoplasm. Prokaryotic cells lack nuclei but still use mRNA to convey a message from the DNA to ribosomes and other cellular equipment that translate the coded information into amino acid sequences. Later in the

▼ **Figure 5.22 Gene expression: DNA → RNA → protein.** In a eukaryotic cell, DNA in the nucleus programs protein production in the cytoplasm by dictating synthesis of messenger RNA (mRNA).



 **BioFlix® Animation: Gene Expression**

book, you'll read about other functions of some recently discovered RNA molecules; the stretches of DNA that direct synthesis of these RNAs are also considered genes (see Concept 18.3).

The Components of Nucleic Acids

Nucleic acids are macromolecules that exist as polymers called **polynucleotides (Figure 5.23a)**. As indicated by the name, each polynucleotide consists of monomers called **nucleotides**. A nucleotide, in general, is composed of three parts: a five-carbon sugar (a pentose), a nitrogen-containing (nitrogenous) base, and one to three phosphate groups (**Figure 5.23b**). The beginning monomer used to build a polynucleotide has three phosphate groups, but two are lost during the polymerization process. The portion of a nucleotide without any phosphate groups is called a *nucleoside*.

To understand the structure of a single nucleotide, let's first consider the nitrogenous bases (**Figure 5.23c**). Each nitrogenous base has one or two rings that include nitrogen atoms. (They are called nitrogenous *bases* because the nitrogen atoms tend to take up H^+ from solution, thus acting as bases.) There are two families of nitrogenous bases: pyrimidines and purines. A **pyrimidine** has one six-membered ring of carbon and nitrogen atoms. The members of the pyrimidine family are cytosine (C), thymine (T), and uracil (U). **Purines** are larger, with a six-membered ring fused to a

five-membered ring. The purines are adenine (A) and guanine (G). The specific pyrimidines and purines differ in the chemical groups attached to the rings. Adenine, guanine, and cytosine are found in both DNA and RNA; thymine is found only in DNA and uracil only in RNA.

Now let's add the sugar to which the nitrogenous base is attached. In DNA the sugar is **deoxyribose**; in RNA it is **ribose** (see Figure 5.23c). The only difference between these two sugars is that deoxyribose lacks an oxygen atom on the second carbon in the ring, hence the name *deoxy*ribose.

So far, we have built a nucleoside (base plus sugar). To complete the construction of a nucleotide, we attach one to three phosphate groups to the 5' carbon of the sugar (the carbon numbers in the sugar include ', the prime symbol; see Figure 5.23b). With one phosphate, this is a nucleoside monophosphate, more often called a nucleotide.

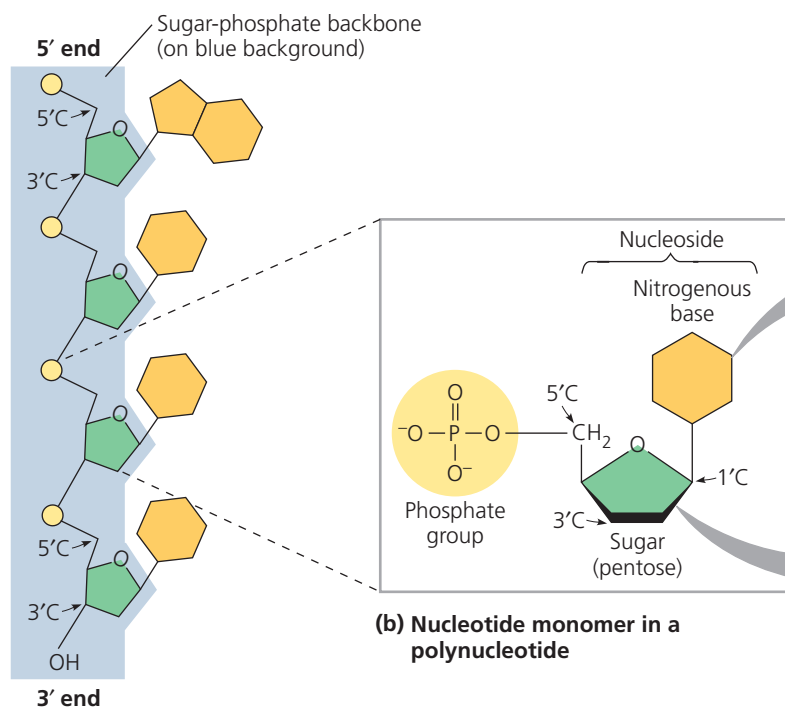
Nucleotide Polymers

The linkage of nucleotides into a polynucleotide involves a dehydration reaction. (You will learn the details in Concept 16.2.) In the polynucleotide, adjacent nucleotides are joined by a phosphodiester linkage, which consists of a phosphate

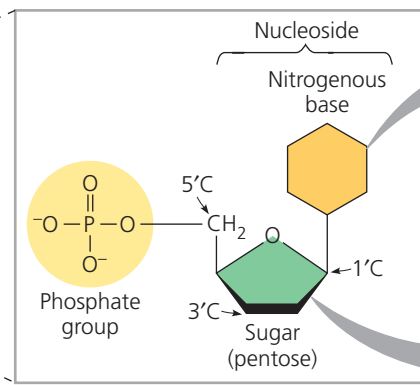
group that links the sugars of two nucleotides. This bonding results in a repeating pattern of sugar-phosphate units called the *sugar-phosphate backbone* (see Figure 5.23a). (Note that the nitrogenous bases are not part of the backbone.) The two free ends of the polymer are distinctly different from each other. One end has a phosphate attached to a 5' carbon, and the other end has a hydroxyl group on a 3' carbon; we refer to these as the *5' end* and the *3' end*, respectively. We can say that a polynucleotide has a built-in directionality along its sugar-phosphate backbone, from 5' to 3', somewhat like a one-way street. The bases are attached all along the sugar-phosphate backbone.

The sequence of bases along a DNA (or mRNA) polymer is unique for each gene and provides very specific information to the cell. Because genes are hundreds to thousands of nucleotides long, the number of possible base sequences is effectively limitless. The information carried by the gene is encoded in its specific sequence of the four DNA bases. For example, the sequence 5'-AGGTAAGT-3' means one thing, whereas the sequence 5'-CGCTTTAAC-3' has a different meaning. (Entire genes, of course, are much longer.) The linear order of bases in a gene specifies the amino acid sequence—the primary structure—of a protein, which in turn specifies that protein's 3-D structure, thus enabling its function in the cell.

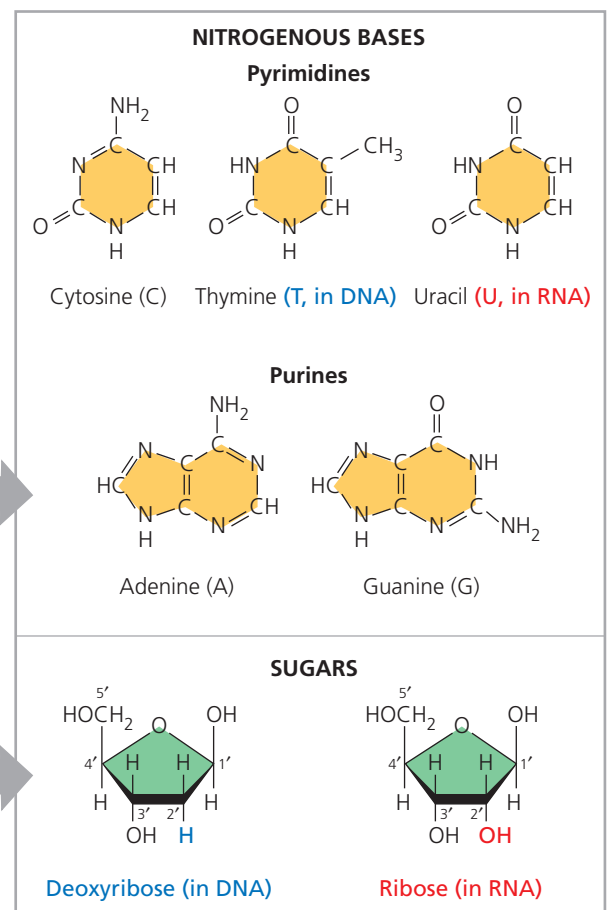
▼ **Figure 5.23 Components of nucleic acids.** (a) A polynucleotide has a sugar-phosphate backbone with variable appendages, the nitrogenous bases. (b) In a polynucleotide, each nucleotide monomer includes a nitrogenous base, a sugar, and a phosphate group. Note that carbon numbers in the sugar include primes ('). (c) A nucleoside includes a nitrogenous base (purine or pyrimidine) and a five-carbon sugar (deoxyribose or ribose).



(a) Polynucleotide, or nucleic acid



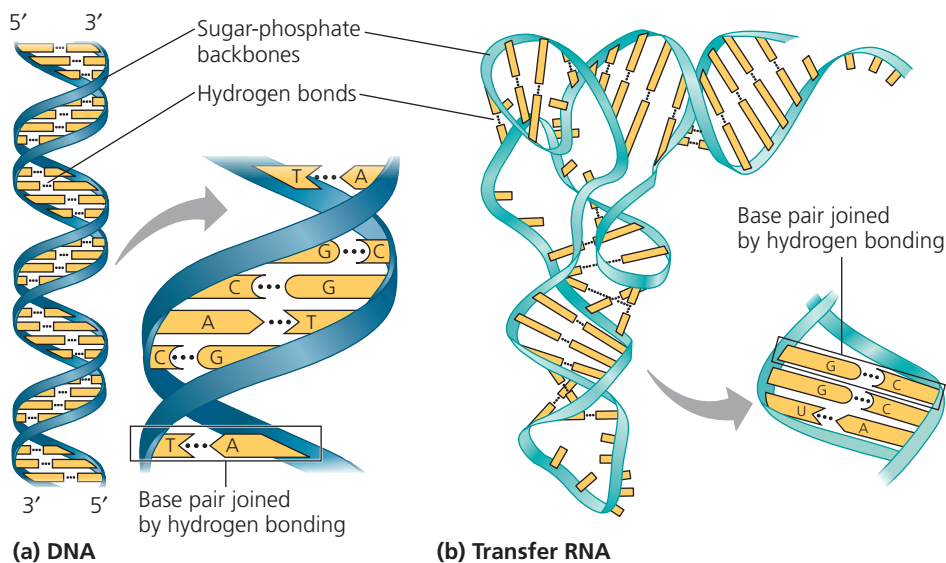
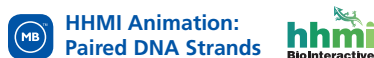
(b) Nucleotide monomer in a polynucleotide



(c) Nucleoside components



► **Figure 5.24 The structures of DNA and tRNA molecules.** (a) The DNA molecule is usually a double helix, with the sugar-phosphate backbones of the antiparallel polynucleotide strands (symbolized here by blue ribbons) on the outside of the helix. Hydrogen bonds between pairs of nitrogenous bases hold the two strands together. As illustrated here with symbolic shapes for the bases, adenine (A) can pair only with thymine (T), and guanine (G) can pair only with cytosine (C). Each DNA strand in this figure is the structural equivalent of the polynucleotide diagrammed in Figure 5.23a. (b) A tRNA molecule has a roughly L-shaped structure due to complementary base pairing of antiparallel stretches of RNA. In RNA, A pairs with U.



The Structures of DNA and RNA Molecules

DNA molecules have two polynucleotides, or “strands,” that wind around an imaginary axis, forming a **double helix** (Figure 5.24a). The two sugar-phosphate backbones run in opposite 5′ → 3′ directions from each other; this arrangement is referred to as **antiparallel**, somewhat like a divided highway. The sugar-phosphate backbones are on the outside of the helix, and the nitrogenous bases are paired in the interior of the helix. The two strands are held together by hydrogen bonds between the paired bases (see Figure 5.24a). Most DNA molecules are very long, with thousands or even millions of base pairs. The one long DNA double helix in a eukaryotic chromosome includes many genes, each one a particular segment of the molecule.

In base pairing, only certain bases in the double helix are compatible with each other. Adenine (A) in one strand always pairs with thymine (T) in the other, and guanine (G) always pairs with cytosine (C). Reading the sequence of bases along one strand of the double helix would tell us the sequence of bases along the other strand. If a stretch of one strand has the base sequence 5′-AGGTC CG-3′, then the base-pairing rules tell us that the same stretch of the other strand must have the sequence 3′-TCCAGGC-5′. The two strands of the double helix are **complementary**, each the predictable counterpart of the other. It is this feature of DNA that makes it possible to generate two identical copies of each DNA molecule in a cell that is preparing to divide. When the cell divides, the copies are distributed to the daughter cells, making them genetically identical to the parent cell. Thus, the structure of DNA accounts for its function of transmitting genetic information whenever a cell reproduces.

RNA molecules, by contrast, exist as single strands. Complementary base pairing can occur, however, between regions of two RNA molecules or even between two stretches of nucleotides in the *same* RNA molecule. In fact, base pairing within an RNA molecule allows it to take on the particular

three-dimensional shape necessary for its function. Consider, for example, the type of RNA called *transfer RNA* (tRNA), which brings amino acids to the ribosome during the synthesis of a polypeptide. A tRNA molecule is about 80 nucleotides in length. Its functional shape results from base pairing between nucleotides where complementary stretches of the molecule can run antiparallel to each other (Figure 5.24b).

Note that in RNA, adenine (A) pairs with uracil (U); thymine (T) is not present in RNA. Another difference between RNA and DNA is that DNA almost always exists as a double helix, whereas RNA molecules are more variable in shape. RNAs are versatile molecules, and many biologists believe RNA may have preceded DNA as the carrier of genetic information in early forms of life (see Concept 25.1).



CONCEPT CHECK 5.5

1. **DRAW IT** ► Go to Figure 5.23a and, for the top three nucleotides, number all the carbons in the sugars, circle the nitrogenous bases, and star the phosphates.
2. **DRAW IT** ► In a DNA double helix, a region along one DNA strand has this sequence of nitrogenous bases: 5′-TAGGCCT-3′. Copy this sequence, and write down its complementary strand, clearly indicating the 5′ and 3′ ends of the complementary strand.

For suggested answers, see Appendix A.

CONCEPT 5.6

Genomics and proteomics have transformed biological inquiry and applications

Experimental work in the first half of the 20th century established the role of DNA as the bearer of genetic information, passed from generation to generation, that

specified the functioning of living cells and organisms. Once the structure of the DNA molecule was described in 1953, and the linear sequence of nucleotide bases was understood to specify the amino acid sequence of proteins, biologists sought to “decode” genes by learning their nucleotide sequences (often called “base sequences”).

The first chemical techniques for *DNA sequencing*, or determining the sequence of nucleotides along a DNA strand, one by one, were developed in the 1970s. Researchers began to study gene sequences, gene by gene, and the more they learned, the more questions they had: How was expression of genes regulated? Genes and their protein products clearly interacted with each other, but how? What was the function, if any, of the DNA that is not part of genes? To fully understand the genetic functioning of a living organism, the entire sequence of the full complement of DNA, the organism’s *genome*, would be most enlightening. In spite of the apparent impracticality of this idea, in the late 1980s several prominent biologists put forth an audacious proposal to launch a project that would sequence the entire human genome—all 3 billion bases of it! This endeavor began in 1990 and was effectively completed in the early 2000s.

An unplanned but profound side benefit of this project—the Human Genome Project—was the rapid development of faster and less expensive methods of sequencing. This trend has continued: The cost for sequencing 1 million bases in 2001, well over \$5,000, has decreased to less than \$0.02 in 2016. And a human genome, the first of which took over 10 years to sequence, could be completed at today’s pace in just a few days (**Figure 5.25**). The number of genomes that have been fully sequenced has burgeoned, generating reams of data and prompting development of **bioinformatics**, the use of computer software and other computational tools that can handle and analyze these large data sets.

The reverberations of these developments have transformed the study of biology and related fields. Biologists often look at problems by analyzing large sets of genes or even comparing whole genomes of different species, an approach called **genomics**. A similar analysis of large sets of proteins, including their sequences, is called **proteomics**. (Protein sequences can be determined either by using biochemical techniques or by translating the DNA sequences that code for them.) These approaches permeate all fields of biology, some examples of which are shown in **Figure 5.26**.



◀ **Figure 5.25**
Automatic DNA sequencing machines and abundant computing power enable rapid sequencing of genes and genomes.

Perhaps the most significant impact of genomics and proteomics on the field of biology as a whole has been their contributions to our understanding of evolution. In addition to confirming evidence for evolution from the study of fossils and characteristics of currently existing species, genomics has helped us tease out relationships among different groups of organisms that had not been resolved by previous types of evidence, and thus infer evolutionary history.

DNA and Proteins as Tape Measures of Evolution

EVOLUTION We are accustomed to thinking of shared traits, such as hair and milk production in mammals, as evidence of shared ancestry. Because DNA carries heritable information in the form of genes, sequences of genes and their protein products document the hereditary background of an organism. The linear sequences of nucleotides in DNA molecules are passed from parents to offspring; these sequences determine the amino acid sequences of proteins. As a result, siblings have greater similarity in their DNA and proteins than do unrelated individuals of the same species.

Given our evolutionary view of life, we can extend this concept of “molecular genealogy” to relationships between species: We would expect two species that appear to be closely related based on anatomical evidence (and possibly fossil evidence) to also share a greater proportion of their DNA and protein sequences than do less closely related species. In fact, that is the case. An example is the comparison of the β polypeptide chain of human hemoglobin with the corresponding hemoglobin polypeptide in other vertebrates. In this chain of 146 amino acids, humans and gorillas differ in just 1 amino acid, while humans and frogs, more distantly related, differ in 67 amino acids. In the **Scientific Skills Exercise**, you can apply this sort of reasoning to additional species. And this conclusion holds true as well when comparing whole genomes: The human genome is 95–98% identical to that of the chimpanzee, but only roughly 85% identical to that of the mouse, a more distant evolutionary relative. Molecular biology has added a new tape measure to the toolkit biologists use to assess evolutionary kinship.

Comparing genomic sequences has practical applications as well. In the **Problem-Solving Exercise**, you can see how this type of genomic analysis can help you detect consumer fraud.

CONCEPT CHECK 5.6

1. How would sequencing the entire genome of an organism help scientists to understand how that organism functioned?
2. Given the function of DNA, why would you expect two species with very similar traits to also have very similar genomes?

For suggested answers, see Appendix A.

Contributions of Genomics and Proteomics to Biology

Nucleotide sequencing and the analysis of large sets of genes and proteins can be done rapidly and inexpensively due to advances in technology and information processing. Taken together, genomics and proteomics have advanced our understanding of biology across many different fields.



Paleontology

New DNA sequencing techniques have allowed decoding of minute quantities of DNA found in ancient tissues from our extinct relatives, the Neanderthals (*Homo neanderthalensis*). Sequencing the Neanderthal genome has informed our understanding of their physical appearance (as in this reconstruction), as well as their relationship with modern humans. (See Figures 34.51 and 34.52.)



Evolution

A major aim of evolutionary biology is to understand the relationships among species, both living and extinct. For example, genome sequence comparisons have identified the hippopotamus as the land mammal sharing the most recent common ancestor with whales. (See Figure 21.20.)



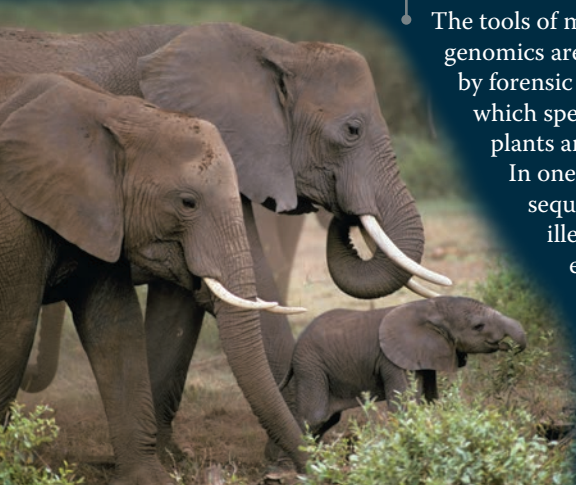
Hippopotamus



Short-finned pilot whale

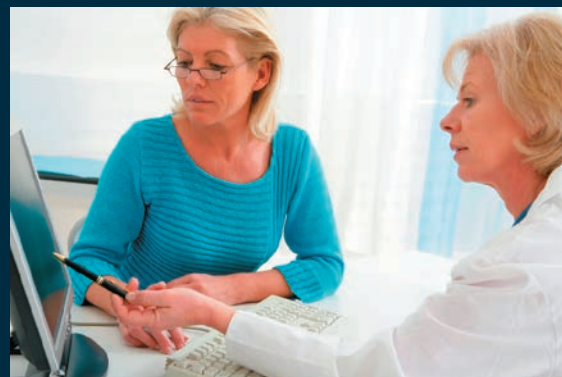
Conservation Biology

The tools of molecular genetics and genomics are increasingly used by forensic ecologists to identify which species of animals and plants are killed illegally. In one case, genomic sequences of DNA from illegal shipments of elephant tusks were used to track down poachers and pinpoint the territory where they were operating. (See Figure 56.9.)



Medical Science

Identifying the genetic basis for human diseases like cancer helps researchers focus their search for potential future treatments. Currently, sequencing the sets of genes expressed in an individual's tumor can allow a more targeted approach to treating the cancer, a type of "personalized medicine." (See Figure 18.27.)



Species Interactions

Most plant species exist in a mutually beneficial partnership with fungi (right) and bacteria associated with the plants' roots; these interactions improve plant growth. Genome sequencing and analysis of gene expression have allowed characterization of plant-associated communities. Such studies will help advance our understanding of such interactions and may improve agricultural practices. (See the Chapter 31 Scientific Skills Exercise and Figure 37.11.)



MAKE CONNECTIONS ► Considering the examples provided here, describe how the approaches of genomics and proteomics help us to address a variety of biological questions.



HHMI Video: The Making of the Fittest: The Birth and Death of Genes (Icefish)



SCIENTIFIC SKILLS EXERCISE



Analyzing Polypeptide Sequence Data

► Human

► Rhesus monkey

► Gibbon

Are Rhesus Monkeys or Gibbons More Closely Related to Humans? In this exercise, you will look at amino acid sequence data for the β polypeptide chain of hemoglobin, often called β -globin. You will then interpret the data to hypothesize whether the monkey or the gibbon is more closely related to humans.

How Such Experiments Are Done Researchers can isolate the polypeptide of interest from an organism and then determine the amino acid sequence. More frequently, the DNA of the relevant gene is sequenced, and the amino acid sequence of the polypeptide is deduced from the DNA sequence of its gene.

Data from the Experiments In the data below, the letters give the sequence of the 146 amino acids in β -globin from humans, rhesus

monkeys, and gibbons. Because a complete sequence would not fit on one line here, the sequences are broken into three segments. The sequences for the three different species are aligned so that you can compare them easily. For example, you can see that for all three species, the first amino acid is V (valine) and the 146th amino acid is H (histidine).

INTERPRET THE DATA

1. Scan the monkey and gibbon sequences, letter by letter, circling any amino acids that do not match the human sequence. (a) How many amino acids differ between the monkey and the human sequences? (b) Between the gibbon and human?
2. For each nonhuman species, what percent of its amino acids are identical to the human sequence of β -globin?

3. Based on these data alone, state a hypothesis for which of these two species is more closely related to humans. What is your reasoning?
4. What other evidence could you use to support your hypothesis?

Data from Human: <http://www.ncbi.nlm.nih.gov/protein/AAA21113.1>; rhesus monkey: <http://www.ncbi.nlm.nih.gov/protein/122634>; gibbon: <http://www.ncbi.nlm.nih.gov/protein/122616>



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Species	Alignment of Amino Acid Sequences of β -globin					
Human	1	VHLTPEEKSA	VTALWGKVV	DEVGGEALGR	LLVYPWTQR	FFESFGDLST
Monkey	1	VHLTPEEKNA	VTTLWGKVV	DEVGGEALGR	LLVYPWTQR	FFESFGDLSS
Gibbon	1	VHLTPEEKSA	VTALWGKVV	DEVGGEALGR	LLVYPWTQR	FFESFGDLST
Human	51	PDAVMGNPKV	KAHGKKVLGA	FSDGLAHLDN	LKGTFAQLSE	LHCDKLHVDP
Monkey	51	PDAVMGNPKV	KAHGKKVLGA	FSDGLNHLDN	LKGTFAQLSE	LHCDKLHVDP
Gibbon	51	PDAVMGNPKV	KAHGKKVLGA	FSDGLAHLDN	LKGTFAQLSE	LHCDKLHVDP
Human	101	ENFRLLGNVL	VCVLAHHFGK	EFTPPVQAAY	QKVVAGVANA	LAHKYH
Monkey	101	ENFKLLGNVL	VCVLAHHFGK	EFTPPVQAAY	QKVVAGVANA	LAHKYH
Gibbon	101	ENFRLLGNVL	VCVLAHHFGK	EFTPPVQAAY	QKVVAGVANA	LAHKYH

PROBLEM-SOLVING EXERCISE

Are you a victim of fish fraud?

When buying salmon, perhaps you prefer the more expensive wild-caught Pacific salmon (*Oncorhynchus* species) over farmed Atlantic salmon (*Salmo salar*). But studies reveal that about 40% of the time, you aren't getting the fish you paid for! Watch the video in the MasteringBiology Study Area for more information.



ABC News Video: Fake Fish in Stores and Restaurants

Instructors: A version of this Problem-Solving Exercise can be assigned in Chapter 5 of MasteringBiology. A more extensive investigation is in Chapter 22 of MasteringBiology.

In this exercise, you will investigate whether a piece of salmon has been fraudulently labeled.

Your Approach The principle guiding your investigation is that DNA sequences from within a species or from closely related species are more similar to each other than are sequences from more distantly related species.

Your Data You've been sold a piece of salmon labeled as coho salmon (*Oncorhynchus kisutch*). To see whether your fish was labeled correctly, you will compare a short DNA sequence from your sample to standard sequences from the same gene for three salmon species. The sequences are:

Sample labeled as *O. kisutch* (coho salmon) 5'-CGGCACCGCCCTAAGTCTCT-3'

Standard sequences

- Sequence for *O. kisutch* (coho salmon) 5'-AGGCACCGCCCTAAGTCTAC-3'
- Sequence for *O. keta* (chum salmon) 5'-AGGCACCGCCCTGAGCCTAC-3'
- Sequence for *Salmo salar* (Atlantic salmon) 5'-CGGCACCGCCCTAAGTCTCT-3'

- Your Analysis**
1. Scan along the standard sequences (*O. kisutch*, *O. keta*, and *S. salar*), base by base, circling any bases that do not match the sequence from your fish sample.
 2. How many bases differ between (a) *O. kisutch* and your fish sample? (b) *O. keta* and the sample? (c) *S. salar* and the sample?
 3. For each standard, what percentage of its bases are identical to your sample?
 4. Based on these data alone, state a hypothesis for the species identity of your sample. What is your reasoning?

5 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 5.1

Macromolecules are polymers, built from monomers (pp. 115–116)

- Large carbohydrates (polysaccharides), proteins, and nucleic acids are **polymers**, which are chains



of **monomers**. The components of lipids vary. Monomers form larger molecules by **dehydration reactions**, in which water molecules are released. Polymers can disassemble by the reverse process, **hydrolysis**. An immense variety of polymers can be built from a small set of monomers.



What is the fundamental basis for the differences between large carbohydrates, proteins, and nucleic acids?

Large Biological Molecules	Components	Examples	Functions
CONCEPT 5.2 Carbohydrates serve as fuel and building material (pp. 116–120) Compare the composition, structure, and function of starch and cellulose. What role do starch and cellulose play in the human body?	Monosaccharide monomer	Monosaccharides: glucose, fructose Disaccharides: lactose, sucrose Polysaccharides: - Cellulose (plants) - Starch (plants) - Glycogen (animals) - Chitin (animals and fungi)	Fuel; carbon sources that can be converted to other molecules or combined into polymers - Strengthens plant cell walls - Stores glucose for energy - Stores glucose for energy - Strengthens exoskeletons and fungal cell walls
CONCEPT 5.3 Lipids are a diverse group of hydrophobic molecules (pp. 120–123) Why are lipids not considered to be polymers or macromolecules?	Glycerol 3 fatty acids Head with P 2 fatty acids Steroid backbone	Triacylglycerols (fats or oils): glycerol + three fatty acids Phospholipids: glycerol + phosphate group + two fatty acids Steroids: four fused rings with attached chemical groups	Important energy source Lipid bilayers of membranes Hydrophilic heads Hydrophobic tails - Component of cell membranes (cholesterol) - Signaling molecules that travel through the body (hormones)
CONCEPT 5.4 Proteins include a diversity of structures, resulting in a wide range of functions (pp. 123–131) Explain the basis for the great diversity of proteins.	Amino acid monomer (20 types)	- Enzymes - Defensive proteins - Storage proteins - Transport proteins - Hormones - Receptor proteins - Motor proteins - Structural proteins	- Catalyze chemical reactions - Protect against disease - Store amino acids - Transport substances - Coordinate organismal responses - Receive signals from outside cell - Function in cell movement - Provide structural support
CONCEPT 5.5 Nucleic acids store, transmit, and help express hereditary information (pp. 132–134) What role does complementary base pairing play in the functions of nucleic acids?	Nitrogenous base Phosphate group Sugar Nucleotide (monomer of a polynucleotide)	DNA: - Sugar = deoxyribose - Nitrogenous bases = C, G, A, T - Usually double-stranded RNA: - Sugar = ribose - Nitrogenous bases = C, G, A, U - Usually single-stranded	Stores hereditary information Various functions in gene expression, including carrying instructions from DNA to ribosomes

CONCEPT 5.6

Genomics and proteomics have transformed biological inquiry and applications (pp. 134–135)

- Recent technological advances in DNA sequencing have given rise to **genomics**, an approach that analyzes large sets of genes or whole genomes, and **proteomics**, a similar approach for large sets of proteins. **Bioinformatics** is the use of computational tools and computer software to analyze these large data sets.
- The more closely two species are related evolutionarily, the more similar their DNA sequences are. DNA sequence data confirm models of evolution based on fossils and anatomical evidence.

? Given the sequences of a particular gene in fruit flies, fish, mice, and humans, predict the relative similarity of the human sequence to that of each of the other species.

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. Construct a table that organizes the following terms, and label the columns and rows.
- | | | |
|-----------------|------------------|-------------------------|
| Monosaccharides | Polypeptides | Phosphodiester linkages |
| Fatty acids | Triacylglycerols | Peptide bonds |
| Amino acids | Polynucleotides | Glycosidic linkages |
| Nucleotides | Polysaccharides | Ester linkages |
9. **DRAW IT** Copy the polynucleotide strand in Figure 5.23a and label the bases G, T, C, and T, starting from the 5' end. Assuming this is a DNA polynucleotide, now draw the complementary strand, using the same symbols for phosphates (circles), sugars (pentagons), and bases. Label the bases. Draw arrows showing the 5' → 3' direction of each strand. Use the arrows to make sure the second strand is antiparallel to the first. *Hint:* After you draw the first strand vertically, turn the paper upside down; it is easier to draw the second strand from the 5' toward the 3' direction as you go from top to bottom.



PRACTICE TEST
goo.gl/iAsVgL

10. **EVOLUTION CONNECTION** Comparisons of amino acid sequences can shed light on the evolutionary divergence of related species. If you were comparing two living species, would you expect all proteins to show the same degree of divergence? Why or why not? Justify your answer.
11. **SCIENTIFIC INQUIRY** Suppose you are provided with a sequence of nucleotides. How would you determine if the sequence is of mRNA, tRNA, or DNA molecules?
12. **WRITE ABOUT A THEME: ORGANIZATION** Proteins, which have diverse functions in a cell, are all polymers of the same kinds of monomers—amino acids. Write a short essay (100–150 words) that discusses how the structure of amino acids allows this one type of polymer to perform so many functions.
13. **SYNTHESIZE YOUR KNOWLEDGE**



Given that the function of egg yolk is to nourish and support the developing chick, explain why egg yolks are so high in fat, protein, and cholesterol.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

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Energy and Life

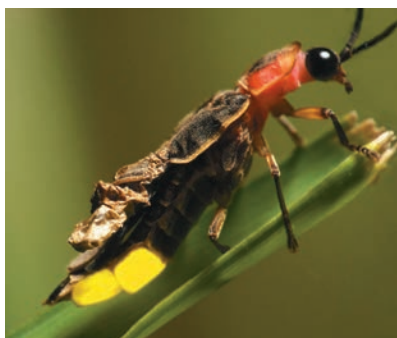


▲ **Figure 6.1** What causes these breaking waves to glow?

KEY CONCEPTS

- 6.1** An organism's metabolism transforms matter and energy, subject to the laws of thermodynamics
- 6.2** The free-energy change of a reaction tells us whether or not the reaction occurs spontaneously
- 6.3** ATP powers cellular work by coupling exergonic reactions to endergonic reactions
- 6.4** Enzymes speed up metabolic reactions by lowering energy barriers
- 6.5** Regulation of enzyme activity helps control metabolism

▼ Firefly



The Energy of Life

The living cell is a chemical factory in miniature, where thousands of reactions occur within a microscopic space. Sugars can be converted to amino acids that are linked together into proteins when needed. Conversely, when food is digested, proteins are dismantled into amino acids that can be converted to sugars. In multicellular organisms, many cells export chemical products that are used in other parts of the organism. The process called cellular respiration drives this cellular economy by extracting the energy stored in sugars and other fuels. Cells apply this energy to perform various types of work, such as the transport of solutes between the cellular interior and the external environment.

In a more exotic example, the ocean waves shown in **Figure 6.1** are brightly illuminated from within by free-floating, single-celled marine organisms called dinoflagellates. These dinoflagellates convert the energy stored in certain organic molecules to light, a process called bioluminescence. Most bioluminescent organisms are found in the oceans, but some exist on land, such as the bioluminescent firefly shown in the small photo. Bioluminescence and other metabolic activities carried out by a cell are precisely coordinated and controlled. In its complexity, its efficiency, and its responsiveness to subtle changes, the cell is peerless as a chemical factory. The concepts of metabolism that you learn in this chapter will help you understand how matter and energy flow during life's processes and how that flow is regulated.

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Get Ready for This Chapter

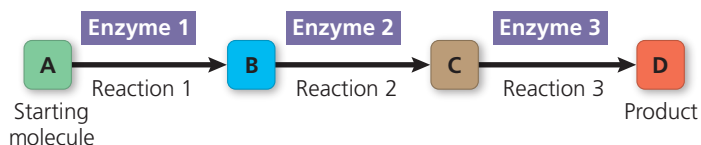
CONCEPT 6.1

An organism's metabolism transforms matter and energy, subject to the laws of thermodynamics

The totality of an organism's chemical reactions is called **metabolism** (from the Greek *metabole*, change). Metabolism is an emergent property of life that arises from orderly interactions between molecules.

Organization of the Chemistry of Life into Metabolic Pathways

We can picture a cell's metabolism as an elaborate road map of the thousands of chemical reactions that occur in a cell, arranged as intersecting metabolic pathways. A **metabolic pathway** begins with a specific molecule, which is then altered in a series of defined steps, resulting in a certain product. Each step of the pathway is catalyzed by a specific enzyme:



There are mechanisms that regulate these enzymes, thus balancing metabolic supply and demand, analogous to the red, yellow, and green stoplights that control the flow of automobile traffic.

Metabolism as a whole manages the material and energy resources of the cell. Some metabolic pathways release energy by breaking down complex molecules to simpler compounds. These degradative processes are called **catabolic pathways**, or breakdown pathways. A major pathway of catabolism is cellular respiration, in which the sugar glucose and other organic fuels are broken down in the presence of oxygen to carbon dioxide and water. (Pathways can have more than one starting molecule and/or product.) Energy that was stored in the organic molecules becomes available to do the work of the cell, such as changing cell shape or transporting solutes. **Anabolic pathways**, in contrast, consume energy to build complicated molecules from simpler ones; they are sometimes called biosynthetic pathways. Examples of anabolism are the synthesis of an amino acid from simpler molecules and the synthesis of a protein from amino acids. Catabolic and anabolic pathways are the “downhill” and “uphill” avenues of the metabolic landscape. Energy released from the downhill reactions of catabolic pathways can be stored and then used to drive the uphill reactions of anabolic pathways.

In this chapter, we will focus on mechanisms common to metabolic pathways. Because energy is fundamental to all metabolic processes, a basic knowledge of energy is necessary to understand how the living cell works. Although we will use

some nonliving examples to study energy, the concepts demonstrated by these examples also apply to **bioenergetics**, the study of how energy flows through living organisms.

Forms of Energy

Energy is the capacity to cause change. In everyday life, energy is important because some forms of energy can be used to do work—that is, to move matter against opposing forces, such as gravity and friction. Put another way, energy is the ability to rearrange a collection of matter. For example, you expend energy to turn the pages of this book, and your cells expend energy in transporting certain substances across membranes. Energy exists in various forms, and the work of life depends on the ability of cells to transform energy from one form to another.

Energy can be associated with the relative motion of objects; this energy is called **kinetic energy**. Moving objects can perform work by imparting motion to other matter: A pool player uses the motion of the cue stick to push the cue ball, which in turn moves the other balls; water gushing through a dam turns turbines; and the contraction of leg muscles pushes bicycle pedals. **Thermal energy** is kinetic energy associated with the random movement of atoms or molecules; thermal energy in transfer from one object to another is called **heat**. Light is also a type of energy that can be harnessed to perform work, such as powering photosynthesis in green plants.

An object not presently moving may still possess energy. Energy that is not kinetic is called **potential energy**; it is energy that matter possesses because of its location or structure. Water behind a dam, for instance, possesses energy because of its altitude above sea level. Molecules possess energy because of the arrangement of electrons in the bonds between their atoms. **Chemical energy** is a term used by biologists to refer to the potential energy available for release in a chemical reaction. Recall that catabolic pathways release energy by breaking down complex molecules. Biologists say that these complex molecules, such as glucose, are high in chemical energy. During a catabolic reaction, some bonds are broken and others are formed, releasing energy and resulting in lower-energy breakdown products. This transformation also occurs in the engine of a car when the hydrocarbons of gasoline react explosively with oxygen, releasing the energy that pushes the pistons and producing exhaust. Although less explosive, a similar reaction of food molecules with oxygen provides chemical energy in biological systems, producing carbon dioxide and water as waste products. Biochemical pathways, carried out in the context of cellular structures, enable cells to release chemical energy from food molecules and use the energy to power life processes.

How is energy converted from one form to another? Consider **Figure 6.2**. The woman climbing the ladder to the diving platform is releasing chemical energy from the food she ate for lunch and using some of that energy to perform

▼ **Figure 6.2** Transformations between potential and kinetic energy.

A diver has more potential energy on the platform than in the water.

Diving converts potential energy to kinetic energy.



Climbing up converts the kinetic energy of muscle movement to potential energy.

A diver has less potential energy in the water than on the platform.

 **Animation: Energy Transformations**

the work of climbing. The kinetic energy of muscle movement is thus being transformed into potential energy due to her increasing height above the water. The man diving is converting his potential energy to kinetic energy, which is then transferred to the water as he enters it, resulting in splashing, noise, and increased movement of water molecules. A small amount of energy is lost as heat due to friction.

Now let's consider the original source of the organic food molecules that provided the necessary chemical energy for these divers to climb the steps. This chemical energy was itself

derived from light energy absorbed by plants during photosynthesis. Organisms are energy transformers.

The Laws of Energy Transformation

The study of the energy transformations that occur in a collection of matter is called **thermodynamics**. Scientists use the word *system* to denote the matter under study; they refer to the rest of the universe—everything outside the system—as the *surroundings*. An *isolated system*, such as that approximated by liquid in a thermos bottle, is unable to exchange either energy or matter with its surroundings outside the thermos. In an *open system*, energy and matter can be transferred between the system and its surroundings. Organisms are open systems. They absorb energy—for instance, light energy or chemical energy in the form of organic molecules—and release heat and metabolic waste products, such as carbon dioxide, to the surroundings. Two laws of thermodynamics govern energy transformations in organisms and all other collections of matter.

The First Law of Thermodynamics

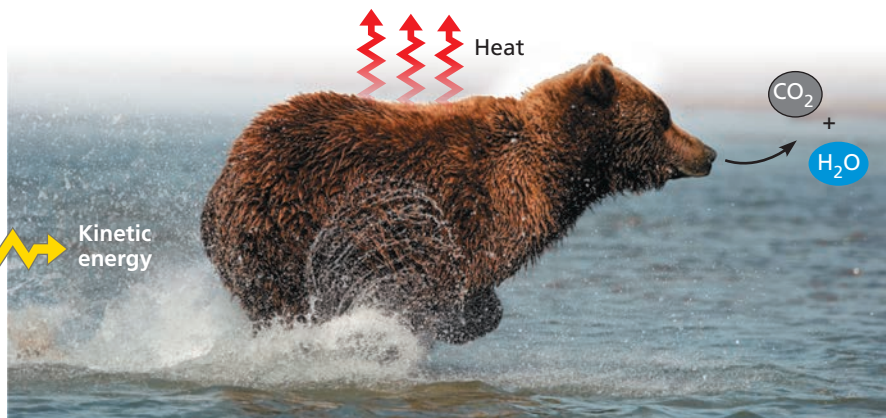
According to the **first law of thermodynamics**, the energy of the universe is constant: *Energy can be transferred and transformed, but it cannot be created or destroyed*. The first law is also known as the *principle of conservation of energy*. The electric company does not make energy, but merely converts it to a form that is convenient for us to use. By converting sunlight to chemical energy, a plant acts as an energy transformer, not an energy producer.

The brown bear in **Figure 6.3a** will convert the chemical energy of the organic molecules in its food to kinetic and other forms of energy as it carries out biological processes. What happens to this energy after it has performed work? The second law of thermodynamics helps to answer this question.

▼ **Figure 6.3** The two laws of thermodynamics.



(a) First law of thermodynamics: Energy can be transferred or transformed but neither created nor destroyed. For example, chemical reactions in this brown bear will convert the chemical (potential) energy in the fish into the kinetic energy of running.



(b) Second law of thermodynamics: Every energy transfer or transformation increases the disorder (entropy) of the universe. For example, as the bear runs, disorder is increased around its body by the release of heat and small molecules that are the by-products of metabolism. A brown bear can run at speeds up to 35 miles per hour (56 km/hr)—as fast as a racehorse.

 **MP3 Tutor: Basic Energy Concepts**

The Second Law of Thermodynamics

If energy cannot be destroyed, why can't organisms simply recycle their energy over and over again? It turns out that during every energy transfer or transformation, some energy becomes unavailable to do work. In most energy transformations, the more usable forms of energy are at least partly converted to thermal energy and released as heat. Only a small fraction of the chemical energy from the food in Figure 6.3a is transformed into the motion of the brown bear shown in **Figure 6.3b**; most is lost as heat, which dissipates rapidly through the surroundings.

In the process of carrying out chemical reactions that perform various kinds of work, living cells unavoidably convert other forms of energy to heat. A system can put this energy to work only when there is a temperature difference that results in thermal energy flowing as heat from a warmer location to a cooler one. If temperature is uniform, as it is in a living cell, then the heat generated during a chemical reaction will simply warm a body of matter, such as the organism. (This can make a room crowded with people uncomfortably warm, as each person is carrying out a multitude of chemical reactions!)

A consequence of the loss of usable energy as heat to the surroundings is that each energy transfer or transformation makes the universe more disordered. We are all familiar with the word “disorder” in the sense of a messy room or a run-down building. The word “disorder” as used by scientists, however, has a specific molecular definition related to how dispersed the energy is in a system, and how many different energy levels are present. For simplicity, we use “disorder” in the following discussion because our common understanding (the messy room) is a good analogy for molecular disorder.

Scientists use a quantity called **entropy** as a measure of molecular disorder, or randomness. The more randomly arranged a collection of matter is, the greater its entropy. We can now state the **second law of thermodynamics**: *Every energy transfer or transformation increases the entropy of the universe.* Although order can increase locally, there is an unstoppable trend toward randomization of the universe as a whole.

The physical disintegration of a system's organized structure is a good analogy for an increase in entropy. For example, you can observe the gradual decay of an unmaintained building over time. Much of the increasing entropy of the universe is more abstract, however, because it takes the form of increasing amounts of heat and less ordered forms of matter. As the bear in Figure 6.3b converts chemical energy to kinetic energy, it is also increasing the disorder of its surroundings by producing heat and small molecules, such as the CO_2 it exhales, that are the breakdown products of food.

The concept of entropy helps us understand why certain processes are energetically favorable and occur on their own. It turns out that if a given process, by itself, leads to an increase in entropy, that process can proceed without requiring an input

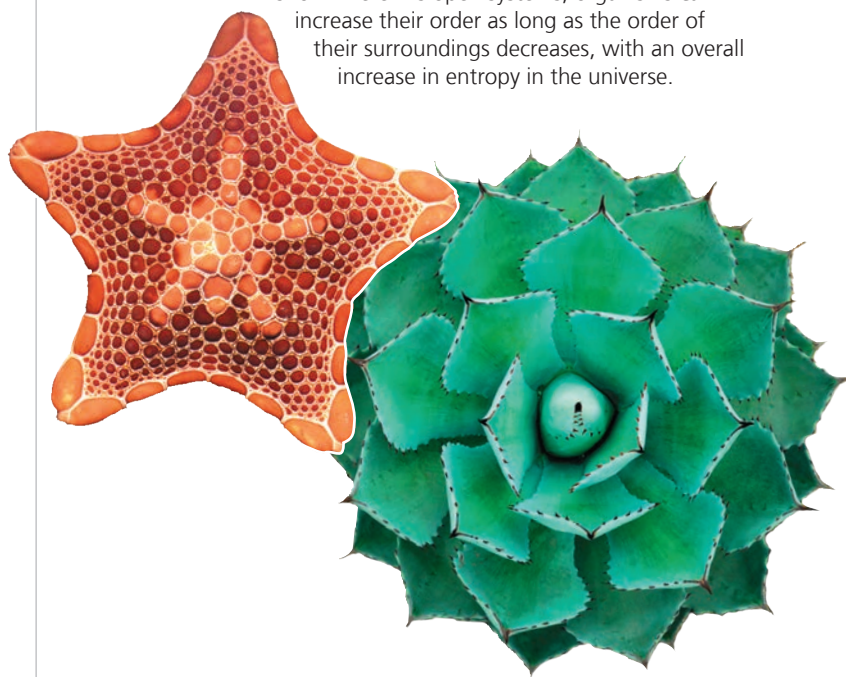
of energy. Such a process is called a **spontaneous process**. Note that as we're using it here, the word *spontaneous* does not imply that the process would occur quickly; rather, the word signifies that it is energetically favorable. (In fact, it may be helpful for you to think of the phrase “energetically favorable” when you read the formal term “spontaneous,” the word favored by chemists.) Some spontaneous processes, such as an explosion, may be virtually instantaneous, while others, such as the rusting of an old car over time, are much slower.

A process that, on its own, leads to a decrease in entropy is said to be nonspontaneous: It will happen only if energy is supplied. We know from experience that certain events occur spontaneously and others do not. For instance, we know that water flows downhill spontaneously but moves uphill only with an input of energy, such as when a machine pumps the water against gravity. Some energy is inevitably lost as heat, increasing entropy in the surroundings, so usage of energy means that a nonspontaneous process also leads to an increase in the entropy of the universe as a whole.

Biological Order and Disorder

Living systems increase the entropy of their surroundings, as predicted by thermodynamic law. It is true that cells create ordered structures from less organized starting materials. For example, simpler molecules are ordered into the more complex structure of an amino acid, and amino acids are ordered into polypeptide chains. At the organismal level as well, complex and beautifully ordered structures result from biological processes that use simpler starting materials (**Figure 6.4**).

▼ **Figure 6.4 Order as a characteristic of life.** Order is evident in the detailed structures of the biscuit star and the agave plant shown here. As open systems, organisms can increase their order as long as the order of their surroundings decreases, with an overall increase in entropy in the universe.



However, an organism also takes in organized forms of matter and energy from the surroundings and replaces them with less ordered forms. For example, an animal obtains starch, proteins, and other complex molecules from the food it eats. As catabolic pathways break these molecules down, the animal releases carbon dioxide and water—small molecules that possess less chemical energy than the food did (see Figure 6.3b). The depletion of chemical energy is accounted for by heat generated during metabolism. On a larger scale, energy flows into most ecosystems in the form of light and exits in the form of heat (see Figure 1.11).

During the early history of life, complex organisms evolved from simpler ancestors. For instance, we can trace the ancestry of the plant kingdom from much simpler organisms called green algae to more complex flowering plants. However, this increase in organization over time in no way violates the second law. The entropy of a particular system, such as an organism, may actually decrease as long as the total entropy of the *universe*—the system plus its surroundings—increases. Thus, organisms are islands of low entropy in an increasingly random universe. The evolution of biological order is perfectly consistent with the laws of thermodynamics.

CONCEPT CHECK 6.1

1. **MAKE CONNECTIONS** ► How does the second law of thermodynamics help explain diffusion, the random thermal motion of particles, across a membrane? (See Figure 8.10.)
2. Describe the forms of energy found in an apple as it grows on a tree, then falls, then is digested by someone who eats it.
3. **WHAT IF?** ► If you place a teaspoon of sugar in the bottom of a glass of water, it will dissolve completely over time. Left longer, eventually the water will disappear and the sugar crystals will reappear. Explain these observations in terms of entropy.

For suggested answers, see Appendix A.

CONCEPT 6.2

The free-energy change of a reaction tells us whether or not the reaction occurs spontaneously

The laws of thermodynamics that we've just discussed apply to the universe as a whole. As biologists, we want to understand the chemical reactions of life—for example, which reactions occur spontaneously and which ones require some input of energy from outside. But how can we know this without assessing the energy and entropy changes in the entire universe for each separate reaction?

Free-Energy Change, ΔG

Recall that the universe is really equivalent to “the system” plus “the surroundings.” In 1878, J. Willard Gibbs, a professor

at Yale, defined a very useful function called the Gibbs free energy of a system (without considering its surroundings), symbolized by the letter G . We'll refer to the Gibbs free energy simply as free energy. **Free energy** is the portion of a system's energy that can perform work when temperature and pressure are uniform throughout the system, as in a living cell. Let's consider how we determine the free-energy change that occurs when a system changes—for example, during a chemical reaction.

The change in free energy, ΔG , can be calculated for a chemical reaction by applying the following equation:

$$\Delta G = \Delta H - T\Delta S$$

This equation uses only properties of the system (the reaction) itself: ΔH symbolizes the change in the system's *enthalpy* (in biological systems, equivalent to total energy); ΔS is the change in the system's entropy; and T is the absolute temperature in Kelvin (K) units ($K = ^\circ C + 273$; see Appendix C).

Using chemical methods, we can measure ΔG for any reaction. (The value will depend on conditions such as pH, temperature, and concentrations of reactants and products.) Once we know the value of ΔG for a process, we can use it to predict whether the process will be spontaneous (that is, whether it is energetically favorable and will occur without an input of energy). More than a century of experiments has shown that only processes with a negative ΔG are spontaneous. For ΔG to be negative, ΔH must be negative (the system gives up enthalpy and H decreases) or $T\Delta S$ must be positive (the system gives up order and S increases), or both: When ΔH and $T\Delta S$ are tallied, ΔG has a negative value ($\Delta G < 0$) for all spontaneous processes. In other words, every spontaneous process decreases the system's free energy, and processes that have a positive or zero ΔG are never spontaneous.

This information is immensely interesting to biologists, for it allows us to predict which kinds of change can happen without an input of energy. Such spontaneous changes can be harnessed to perform work. This principle is very important in the study of metabolism, where a major goal is to determine which reactions can supply energy for cellular work.

Free Energy, Stability, and Equilibrium

As we saw in the previous section, when a process occurs spontaneously in a system, we can be sure that ΔG is negative. Another way to think of ΔG is to realize that it represents the difference between the free energy of the final state and the free energy of the initial state:

$$\Delta G = G_{\text{final state}} - G_{\text{initial state}}$$

Thus, ΔG can be negative only when the process involves a loss of free energy during the change from initial state to final state. Because it has less free energy, the system in its final state is less likely to change and is therefore more stable than it was previously.

We can think of free energy as a measure of a system's instability—its tendency to change to a more stable state. Unstable systems (higher G) tend to change in such a way that they become more stable (lower G). For example, a diver on top of a platform is less stable (more likely to fall) than when floating in the water; a drop of concentrated dye is less stable (more likely to disperse) than when the dye is spread randomly through the liquid; and a glucose molecule is less stable (more likely to break down) than the simpler molecules into which it can be split (**Figure 6.5**). Unless something prevents it, each of these systems will move toward greater stability: The diver falls, the solution becomes uniformly colored, and the glucose molecule is broken down into smaller molecules.

Another term that describes a state of maximum stability is *equilibrium*, which you learned about in Concept 2.4 in connection with chemical reactions. There is an important relationship between free energy and equilibrium, including chemical equilibrium. Recall that most chemical reactions are reversible and proceed to a point at which the forward and backward reactions occur at the same rate. The reaction is then said to be at chemical equilibrium, and there is no further net change in the relative concentration of products and reactants.

As a reaction proceeds toward equilibrium, the free energy of the mixture of reactants and products decreases. Free energy increases when a reaction is somehow pushed away from equilibrium, perhaps by removing some of the

products (and thus changing their concentration relative to that of the reactants). For a system at equilibrium, G is at its lowest possible value in that system. We can think of the equilibrium state as a free-energy valley. Any change from the equilibrium position will have a positive ΔG and will not be spontaneous. For this reason, systems never spontaneously move away from equilibrium. Because a system at equilibrium cannot spontaneously change, it can do no work. *A process is spontaneous and can perform work only when it is moving toward equilibrium.*

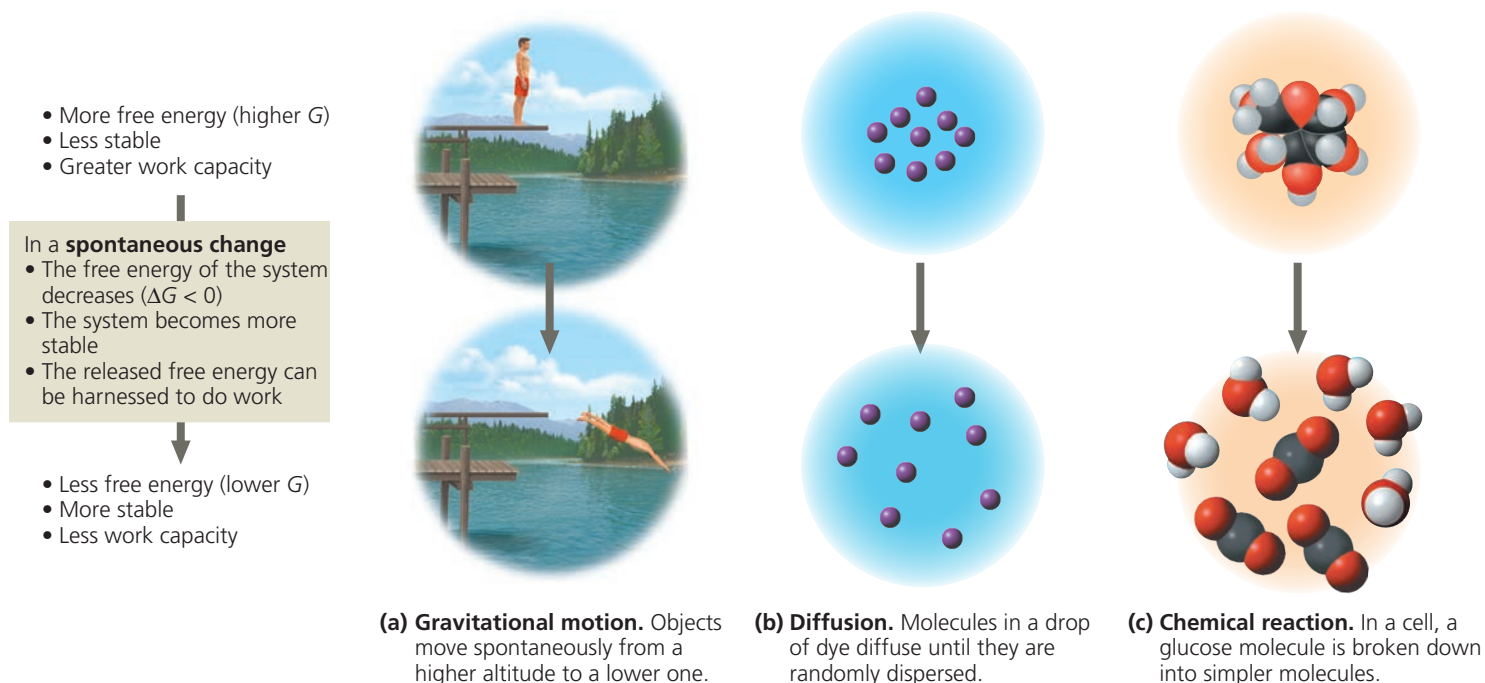
Free Energy and Metabolism

We can now apply the free-energy concept more specifically to the chemistry of life's processes.

Exergonic and Endergonic Reactions in Metabolism

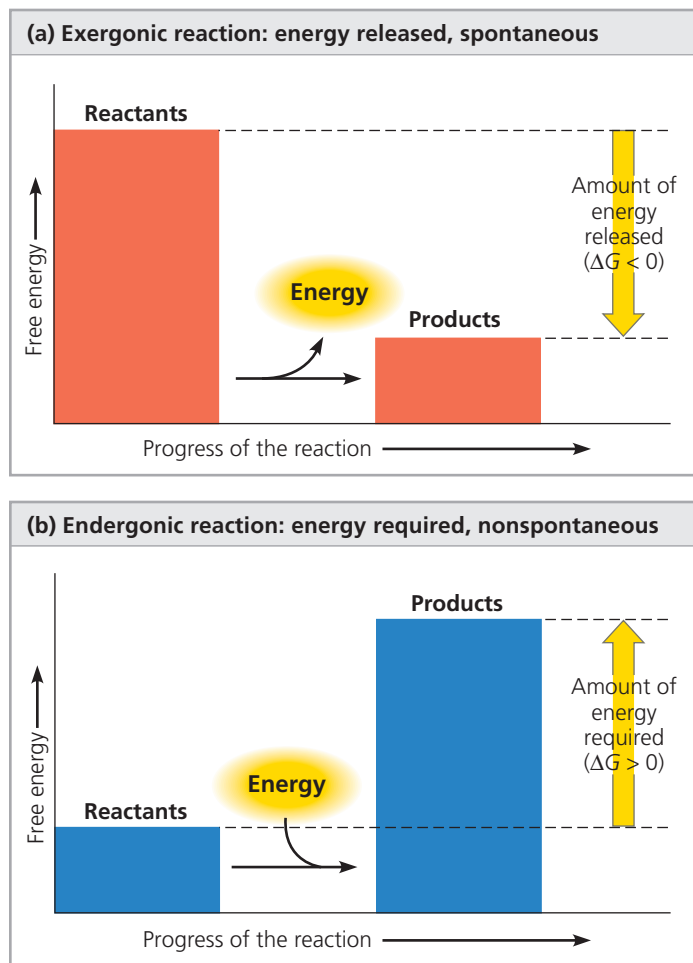
Based on their free-energy changes, chemical reactions can be classified as either exergonic ("energy outward") or endergonic ("energy inward"). An **exergonic reaction** proceeds with a net release of free energy (**Figure 6.6a**). Because the chemical mixture loses free energy (G decreases), ΔG is negative for an exergonic reaction. Using ΔG as a standard for spontaneity, exergonic reactions are those that occur spontaneously. (Remember, the word *spontaneous* implies that

▼ Figure 6.5 The relationship of free energy to stability, work capacity, and spontaneous change. Unstable systems (top) are rich in free energy, G . They have a tendency to change spontaneously to a more stable state (bottom), and it is possible to harness this "downhill" change to perform work.



MAKE CONNECTIONS ▶ Compare the redistribution of molecules shown in (b) to the transport of hydrogen ions (H^+) across a membrane by a proton pump, creating a difference in the concentration of H^+ ions on either side of the membrane, as shown in Figure 8.17. Which process(es) result(s) in higher free energy? Which system(s) can do work?

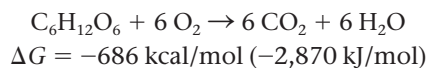
▼ **Figure 6.6** Free energy changes (ΔG) in exergonic and endergonic reactions.



Animation: Exergonic and Endergonic Reactions

it is energetically favorable, not that it will occur rapidly.) The magnitude of ΔG for an exergonic reaction represents the maximum amount of work the reaction can perform.* The greater the decrease in free energy, the greater the amount of work that can be done.

We can use the overall reaction for cellular respiration as an example:



For each mole (180 g) of glucose broken down by respiration under what are called “standard conditions” (1 M of each reactant and product, 25°C, pH 7), 686 kcal (2,870 kJ) of energy is made available for work. Because energy must be conserved, the chemical *products* of respiration store 686 kcal less free energy per mole than the *reactants*. The products are,

*The word *maximum* qualifies this statement, because some of the free energy is released as heat and cannot do work. Therefore, ΔG represents a theoretical upper limit of available energy.

in a sense, the spent exhaust of a process that tapped the free energy stored in the bonds of the sugar molecules.

It is important to realize that the breaking of bonds does not release energy; on the contrary, as you will soon see, it requires energy. The phrase “energy stored in bonds” is shorthand for the potential energy that can be released when new bonds are formed after the original bonds break, as long as the products are of lower free energy than the reactants.

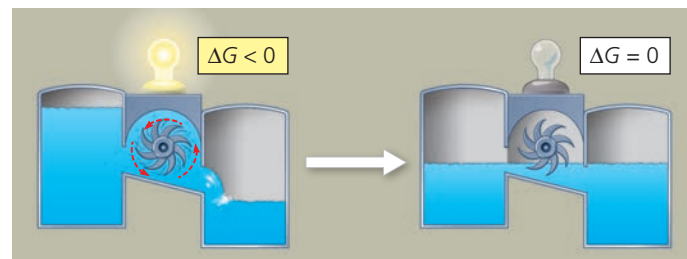
An **endergonic reaction** is one that absorbs free energy from its surroundings (Figure 6.6b). Because this kind of reaction essentially *stores* free energy in molecules (G increases), ΔG is positive. Such reactions are nonspontaneous, and the magnitude of ΔG is the quantity of energy required to drive the reaction. If a chemical process is exergonic (downhill), releasing energy in one direction, then the reverse process must be endergonic (uphill), using energy. A reversible process cannot be downhill in both directions. If $\Delta G = -686 \text{ kcal/mol}$ for respiration, which converts glucose and oxygen to carbon dioxide and water, then the reverse process—the conversion of carbon dioxide and water to glucose and oxygen—must be strongly endergonic, with $\Delta G = +686 \text{ kcal/mol}$. Such a reaction would never happen by itself.

How, then, do plants make the sugar that organisms use for energy? Plants get the required energy—686 kcal to make a mole of glucose—from the environment by capturing light and converting its energy to chemical energy. Next, in a long series of exergonic steps, they gradually spend that chemical energy to assemble glucose molecules.

Equilibrium and Metabolism

Reactions in an isolated system eventually reach equilibrium and can then do no work, as illustrated by the isolated hydroelectric system in Figure 6.7. The chemical reactions of metabolism are reversible, and they, too, would reach equilibrium if they occurred in the isolation of a test tube. Because systems at equilibrium are at a minimum of G and can do no work, a cell that has reached metabolic equilibrium is dead! *The fact that metabolism as a whole is never at equilibrium is one of the defining features of life.*

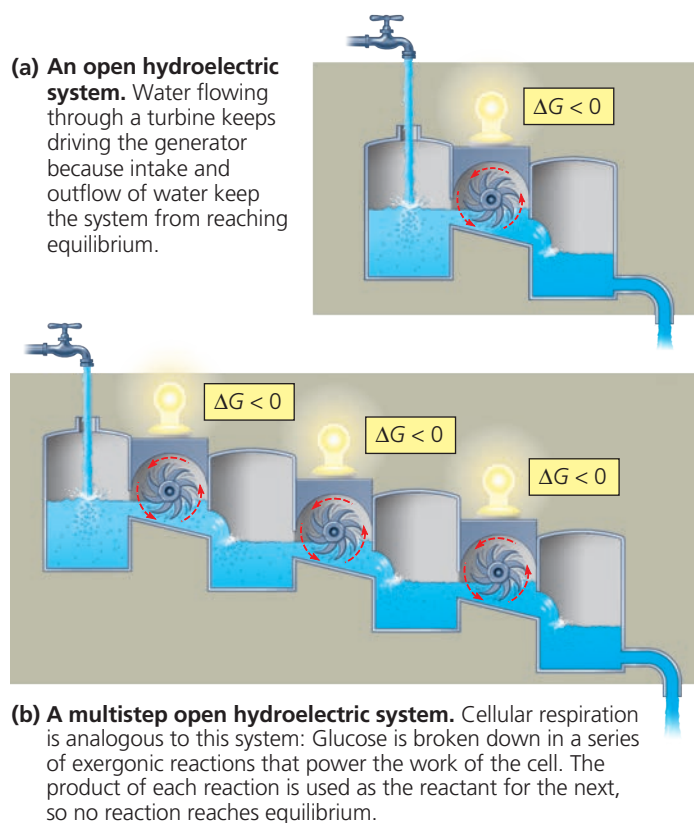
▼ **Figure 6.7** Equilibrium and work in an isolated hydroelectric system. Water flowing downhill turns a turbine that drives a generator providing electricity to a lightbulb, but only until the system reaches equilibrium.



Like most systems, a living cell is not in equilibrium. The constant flow of materials in and out of the cell keeps the metabolic pathways from ever reaching equilibrium, and the cell continues to do work throughout its life. This principle is illustrated by the open (and more realistic) hydroelectric system in **Figure 6.8a**. However, unlike this simple system in which water flowing downhill turns a single turbine, a catabolic pathway in a cell releases free energy in a series of reactions. An example is cellular respiration, illustrated by analogy in **Figure 6.8b**. Some of the reversible reactions of respiration are constantly “pulled” in one direction—that is, they are kept out of equilibrium. The key to maintaining this lack of equilibrium is that the product of a reaction does not accumulate but instead becomes a reactant in the next step; finally, waste products are expelled from the cell. The overall sequence of reactions is kept going by the huge free-energy difference between glucose and oxygen at the top of the energy “hill” and carbon dioxide and water at the “downhill” end. As long as our cells have a steady supply of glucose or other fuels and oxygen and are able to expel waste products to the surroundings, their metabolic pathways never reach equilibrium and can continue to do the work of life.

Stepping back to look at the big picture, we can see once again how important it is to think of organisms as open systems. Sunlight provides a daily source of free energy for an ecosystem’s plants and other photosynthetic organisms.

▼ **Figure 6.8** Equilibrium and work in open systems.



Animals and other nonphotosynthetic organisms in an ecosystem must have a source of free energy in the form of the organic products of photosynthesis. Now that we have applied the free-energy concept to metabolism, we are ready to see how a cell actually performs the work of life.

CONCEPT CHECK 6.2

1. Cellular respiration uses glucose and oxygen, which have high levels of free energy, and releases CO_2 and water, which have low levels of free energy. Is cellular respiration spontaneous or not? Is it exergonic or endergonic? What happens to the energy released from glucose?
2. **VISUAL SKILLS** ► How would the processes of catabolism and anabolism relate to Figure 6.5c?
3. **WHAT IF?** ► Some nighttime partygoers wear glow-in-the-dark necklaces. The necklaces start glowing once they are “activated” by snapping the necklace in a way that allows two chemicals to react and emit light in the form of chemiluminescence. Is the chemical reaction exergonic or endergonic? Explain your answer.

For suggested answers, see Appendix A.

CONCEPT 6.3

ATP powers cellular work by coupling exergonic reactions to endergonic reactions

A cell does three main kinds of work:

- **Chemical work**, the pushing of endergonic reactions that would not occur spontaneously, such as the synthesis of polymers from monomers (chemical work will be discussed further here; examples are shown in Chapters 10 and 11)
- **Transport work**, the pumping of substances across membranes against the direction of spontaneous movement (which will be discussed in Chapter 8)
- **Mechanical work**, such as the beating of cilia (see Concept 7.6), the contraction of muscle cells, and the movement of chromosomes during cellular reproduction

A key feature in the way cells manage their energy resources to do this work is **energy coupling**, the use of an exergonic process to drive an endergonic one. ATP is responsible for mediating most energy coupling in cells, and in most cases it acts as the immediate source of energy that powers cellular work.

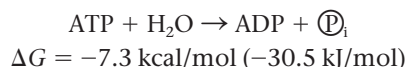
 **Animation: Energy Coupling**

The Structure and Hydrolysis of ATP

ATP (adenosine triphosphate) was introduced when we discussed the phosphate group as a functional group (see Concept 4.3). ATP contains the sugar ribose, with the

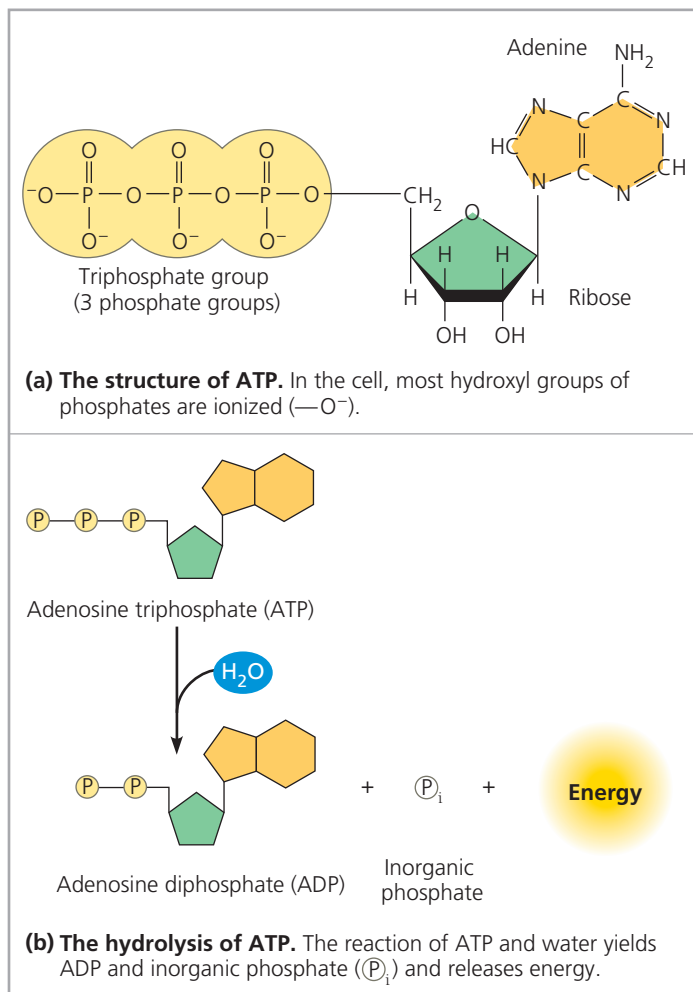
nitrogenous base adenine and a chain of three phosphate groups (the triphosphate group) bonded to it (**Figure 6.9a**). In addition to its role in energy coupling, ATP is also one of the nucleoside triphosphates used to make RNA (see Figure 5.23).

The bonds between the phosphate groups of ATP can be broken by hydrolysis. When the terminal phosphate bond is broken by addition of a water molecule, a molecule of inorganic phosphate (HOPO_3^{2-} , abbreviated P_i throughout this book) leaves the ATP, which becomes adenosine diphosphate, or ADP (**Figure 6.9b**). The reaction is exergonic and releases 7.3 kcal of energy per mole of ATP hydrolyzed:



This is the free-energy change measured under standard conditions. In the cell, conditions do not conform to standard

▼ **Figure 6.9 The structure and hydrolysis of adenosine triphosphate (ATP).** Throughout this book, the chemical structure of the triphosphate group seen in (a) will be represented by the three joined yellow circles shown in (b).



Animation: The Structure of ATP
Animation: Space-Filling Model of ATP
Animation: Stick Model of ATP

conditions, primarily because reactant and product concentrations differ from 1 M. For example, when ATP hydrolysis occurs under cellular conditions, the actual ΔG is about -13 kcal/mol , 78% greater than the energy released by ATP hydrolysis under standard conditions.

Because their hydrolysis releases energy, the phosphate bonds of ATP are sometimes referred to as high-energy phosphate bonds, but the term is misleading. The phosphate bonds of ATP are not unusually strong bonds, as “high-energy” may imply; rather, the reactants (ATP and water) themselves have high energy relative to the energy of the products (ADP and P_i). The release of energy during the hydrolysis of ATP comes from the chemical change of the system to a state of lower free energy, not from the phosphate bonds themselves.

ATP is useful to the cell because the energy it releases on losing a phosphate group is somewhat greater than the energy most other molecules could deliver. But why does this hydrolysis release so much energy? If we reexamine the ATP molecule in Figure 6.9a, we can see that all three phosphate groups are negatively charged. These like charges are crowded together, and their mutual repulsion contributes to the instability of this region of the ATP molecule. The triphosphate tail of ATP is the chemical equivalent of a compressed spring.

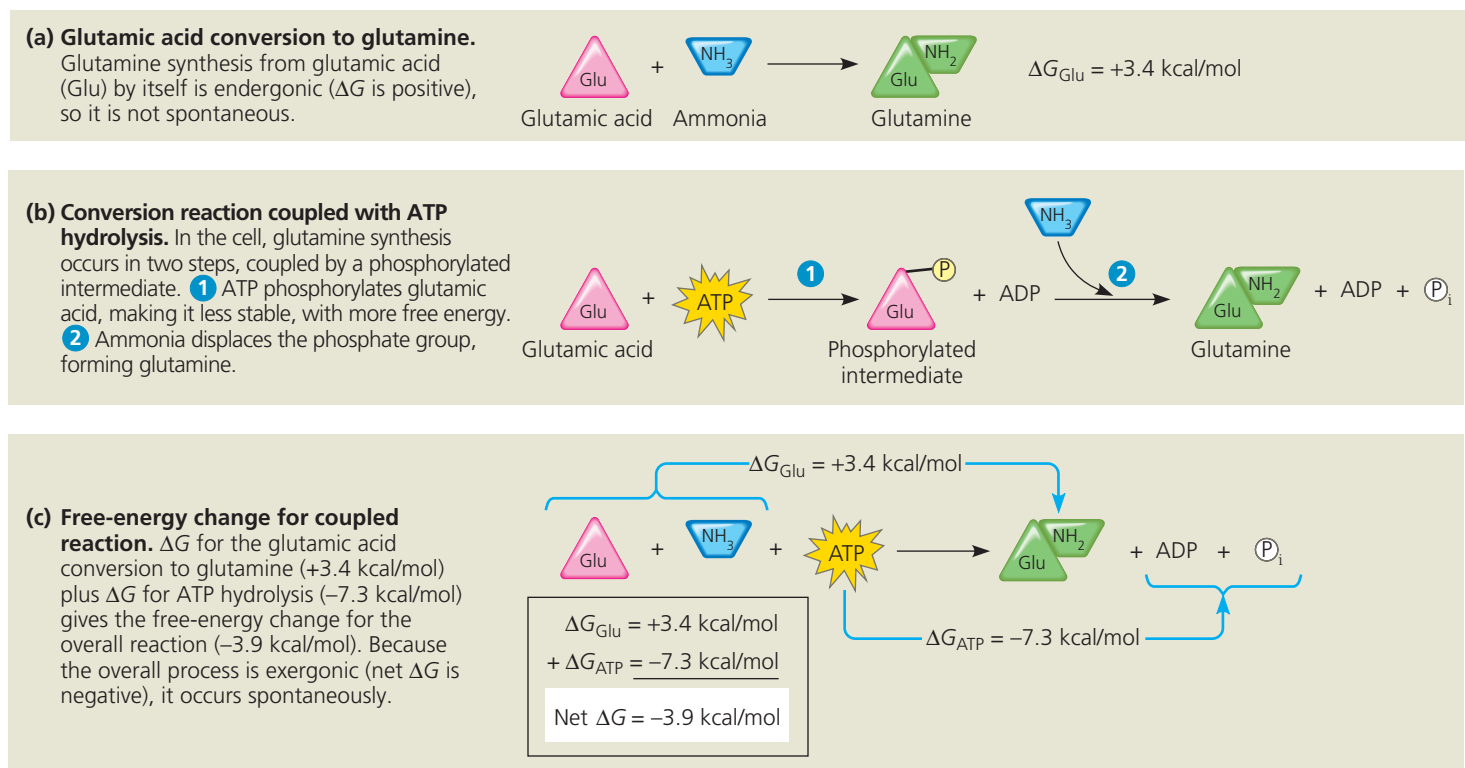
How the Hydrolysis of ATP Performs Work

When ATP is hydrolyzed in a test tube, the release of free energy merely heats the surrounding water. In an organism, this same generation of heat can sometimes be beneficial. For instance, the process of shivering uses ATP hydrolysis during muscle contraction to warm the body. In most cases in the cell, however, the generation of heat alone would be an inefficient (and potentially dangerous) use of a valuable energy resource. Instead, the cell’s proteins harness the energy released during ATP hydrolysis in several ways to perform the three types of cellular work—chemical, transport, and mechanical.

For example, with the help of specific enzymes, the cell is able to use the energy released by ATP hydrolysis directly to drive chemical reactions that, by themselves, are endergonic. If the ΔG of an endergonic reaction is less than the amount of energy released by ATP hydrolysis, then the two reactions can be coupled so that, overall, the coupled reactions are exergonic. This usually involves phosphorylation, the transfer of a phosphate group from ATP to some other molecule, such as the reactant. The recipient molecule with the phosphate group covalently bonded to it is then called a **phosphorylated intermediate**. The key to coupling exergonic and endergonic reactions is the formation of this phosphorylated intermediate, which is more

▼ Figure 6.10 How ATP drives chemical work: Energy coupling using ATP hydrolysis.

In this example, the exergonic process of ATP hydrolysis is used to drive an endergonic process—the cellular synthesis of the amino acid glutamine from glutamic acid and ammonia.



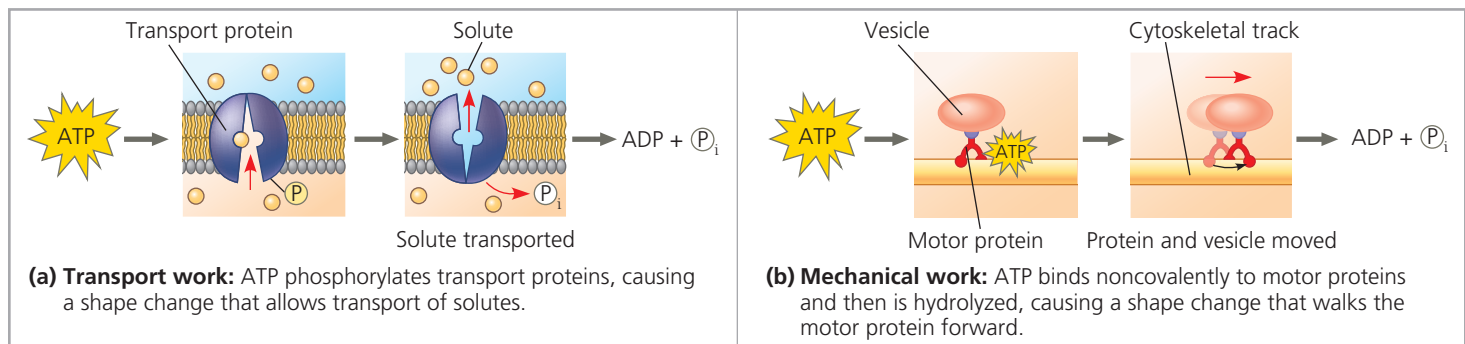
MAKE CONNECTIONS ▶ Referring to Figure 5.14, explain why glutamine (Gln) is diagrammed as a glutamic acid (Glu) with an amino group attached.

reactive (less stable, with more free energy) than the original unphosphorylated molecule (Figure 6.10).

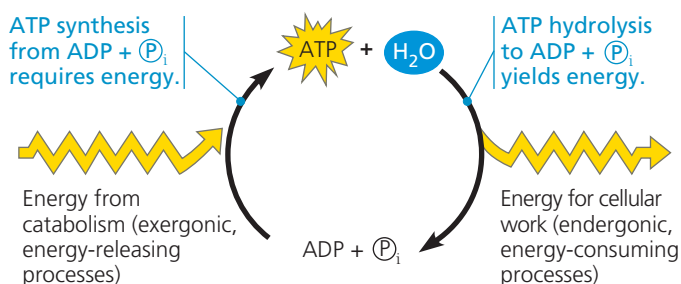
Transport and mechanical work in the cell are also nearly always powered by the hydrolysis of ATP. In these cases, ATP hydrolysis leads to a change in a protein's shape and often its ability to bind another molecule. Sometimes this occurs via a phosphorylated intermediate, as seen for the transport protein in Figure 6.11a. In most instances of mechanical work involving motor proteins "walking" along the cytoskeleton,

a fibrous network in the cytoplasm (Figure 6.11b), a cycle occurs in which ATP is first bound noncovalently to the motor protein. Next, ATP is hydrolyzed, releasing ADP and P_i . Another ATP molecule can then bind. At each stage, the motor protein changes its shape and ability to bind the cytoskeleton, resulting in movement of the protein along the cytoskeletal track. Phosphorylation and dephosphorylation promote crucial protein shape changes during many other important cellular processes as well.

▼ Figure 6.11 How ATP drives transport and mechanical work. ATP hydrolysis causes changes in the shapes and binding affinities of proteins. This can occur either (a) directly, by phosphorylation, as shown for a membrane protein carrying out active transport of a solute (see also Figure 8.15), or (b) indirectly, via noncovalent binding of ATP and its hydrolytic products, as is the case for motor proteins that move vesicles (a type of organelle) along cytoskeletal "tracks" in the cell (see also Figure 7.21).



▼ **Figure 6.12 The ATP cycle.** Energy released by breakdown reactions (catabolism) in the cell is used to phosphorylate ADP, regenerating ATP. Chemical potential energy stored in ATP drives most cellular work.

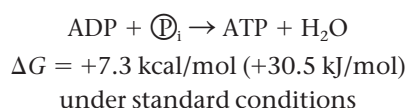


Animation: Metabolism Overview

The Regeneration of ATP

An organism at work uses ATP continuously, but ATP is a renewable resource that can be regenerated by the addition of phosphate to ADP (**Figure 6.12**). The free energy required to phosphorylate ADP comes from exergonic breakdown reactions (catabolism) in the cell. This shuttling of inorganic phosphate and energy is called the ATP cycle, and it couples the cell's energy-yielding (exergonic) processes to the energy-consuming (endergonic) ones. The ATP cycle proceeds at an astonishing pace. For example, a working muscle cell recycles its entire pool of ATP in less than a minute. That turnover represents 10 million molecules of ATP consumed and regenerated per second per cell. If ATP could not be regenerated by the phosphorylation of ADP, humans would use up nearly their body weight in ATP each day.

Because both directions of a reversible process cannot be downhill, the regeneration of ATP is necessarily endergonic:



Since ATP formation from ADP and P_i is not spontaneous, free energy must be spent to make it occur. Catabolic (exergonic) pathways, especially cellular respiration, provide the energy for the endergonic process of making ATP. Plants also use light energy to produce ATP. Thus, the ATP cycle is a revolving door through which energy passes during its transfer from catabolic to anabolic pathways.

CONCEPT CHECK 6.3

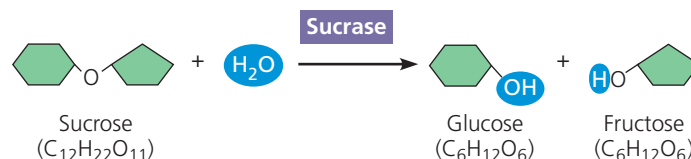
1. How does ATP typically transfer energy from an exergonic to an endergonic reaction in the cell?
2. Which of the following has more free energy: glutamic acid + ammonia + ATP or glutamine + ADP + P_i? Explain your answer.

For suggested answers, see Appendix A.

CONCEPT 6.4

Enzymes speed up metabolic reactions by lowering energy barriers

The laws of thermodynamics tell us what will and will not happen under given conditions but say nothing about the rate of these processes. A spontaneous chemical reaction occurs without any requirement for outside energy, but it may occur so slowly that it is imperceptible. For example, even though the hydrolysis of sucrose (table sugar) to glucose and fructose is exergonic, occurring spontaneously with a release of free energy ($\Delta G = -7 \text{ kcal/mol}$), a solution of sucrose dissolved in sterile water will sit for years at room temperature with no appreciable hydrolysis. However, if we add a small amount of the enzyme sucrase to the solution, then all the sucrose may be hydrolyzed within seconds, as shown here:



How does the enzyme do this?

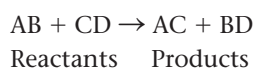
An **enzyme** is a macromolecule that acts as a **catalyst**, a chemical agent that speeds up a reaction without being consumed by the reaction. In this chapter, we are focusing on enzymes that are proteins. (Some RNA molecules, called ribozymes, can function as enzymes; these will be discussed in Concepts 17.3 and 25.1.) Without regulation by enzymes, chemical traffic through the pathways of metabolism would become terribly congested because many chemical reactions would take such a long time. In the next two sections, we will see why spontaneous reactions can be slow and how an enzyme changes the situation.

The Activation Energy Barrier

Every chemical reaction between molecules involves both bond breaking and bond forming. For example, the hydrolysis of sucrose involves breaking the bond between glucose and fructose and one of the bonds of a water molecule and then forming two new bonds, as shown above. Changing one molecule into another generally involves contorting the starting molecule into a highly unstable state before the reaction can proceed. This contortion can be compared to the bending of a metal key ring when you pry it open to add a new key. The key ring is highly unstable in its opened form but returns to a stable state once the key is threaded all the way onto the ring. To reach the contorted state where bonds can change, reactant molecules must absorb energy from their surroundings. When the new bonds of the product molecules form, energy is released as heat, and the molecules return to stable shapes with lower energy than the contorted state.

The initial investment of energy for starting a reaction—the energy required to contort the reactant molecules so the bonds can break—is known as the *free energy of activation*, or **activation energy**, abbreviated E_A in this book. We can think of activation energy as the amount of energy needed to push the reactants to the top of an energy barrier, or uphill, so that the “downhill” part of the reaction can begin. Activation energy is often supplied by heat in the form of thermal energy that the reactant molecules absorb from the surroundings. The absorption of thermal energy accelerates the reactant molecules, so they collide more often and more forcefully. It also agitates the atoms within the molecules, making the breakage of bonds more likely. When the molecules have absorbed enough energy for the bonds to break, the reactants are in an unstable condition known as the *transition state*.

Figure 6.13 graphs the energy changes for a hypothetical exergonic reaction that swaps portions of two reactant molecules:



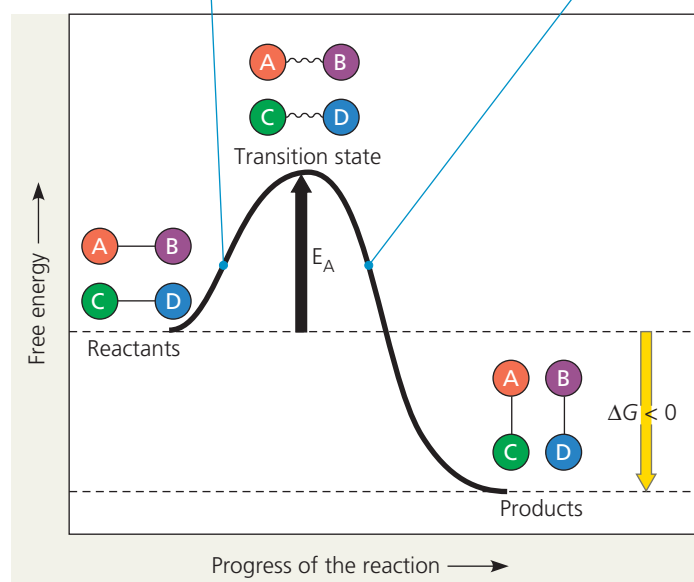
The activation of the reactants is represented by the uphill portion of the graph, in which the free-energy content of

▼ Figure 6.13 Energy profile of an exergonic reaction.

The “molecules” are hypothetical, with A, B, C, and D representing portions of the molecules. Thermodynamically, this is an exergonic reaction, with a negative ΔG , and the reaction occurs spontaneously. However, the activation energy (E_A) provides a barrier that determines the rate of the reaction.

The reactants AB and CD must absorb enough energy from the surroundings to reach the unstable transition state, where bonds can break.

After bonds have broken, new bonds form, releasing energy to the surroundings.



DRAW IT ► Graph the progress of an endergonic reaction in which EF and GH form products EG and FH, assuming that the reactants must pass through a transition state.

the reactant molecules is increasing. At the summit, when energy equivalent to E_A has been absorbed, the reactants are in the transition state: They are activated, and their bonds can be broken. As the atoms then settle into their new, more stable bonding arrangements, energy is released to the surroundings. This corresponds to the downhill part of the curve, which shows the loss of free energy by the molecules. The overall decrease in free energy means that E_A is repaid with dividends, as the formation of new bonds releases more energy than was invested in the breaking of old bonds.

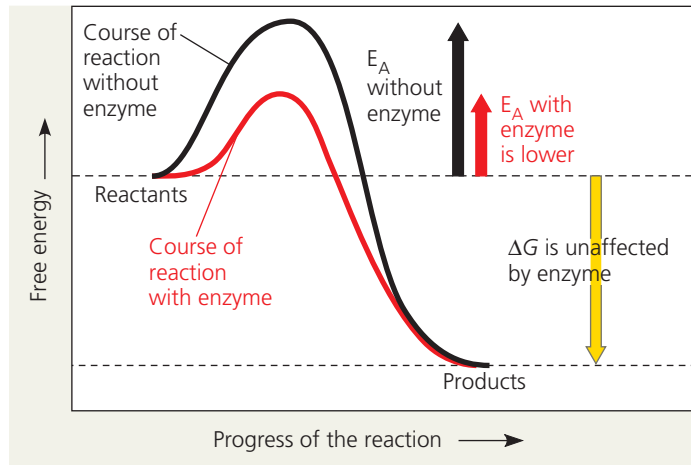
The reaction shown in Figure 6.13 is exergonic and occurs spontaneously ($\Delta G < 0$). However, the activation energy provides a barrier that determines the rate of the reaction. The reactants must absorb enough energy to reach the top of the activation energy barrier before the reaction can occur. For some reactions, E_A is modest enough that even at room temperature there is sufficient thermal energy for many of the reactant molecules to reach the transition state in a short time. In most cases, however, E_A is so high and the transition state is reached so rarely that the reaction will hardly proceed at all. In these cases, the reaction will occur at a noticeable rate only if energy is provided, usually by heat. For example, the reaction of gasoline and oxygen is exergonic and will occur spontaneously, but energy is required for the molecules to reach the transition state and react. Only when the spark plugs fire in an automobile engine can there be the explosive release of energy that pushes the pistons. Without a spark, a mixture of gasoline hydrocarbons and oxygen will not react because the E_A barrier is too high.

How Enzymes Speed Up Reactions

Proteins, DNA, and other complex cellular molecules are rich in free energy and have the potential to decompose spontaneously; that is, the laws of thermodynamics favor their breakdown. These molecules only persist because at temperatures typical for cells, few molecules can make it over the hump of activation energy. The barriers for selected reactions must occasionally be surmounted, however, for cells to carry out the processes needed for life. Heat can increase the rate of a reaction by allowing reactants to attain the transition state more often, but this would not work well in biological systems. First, high temperature denatures proteins and kills cells. Second, heat would speed up *all* reactions, not just those that are needed. Instead of heat, organisms carry out **catalysis**, a process by which a catalyst (for example, an enzyme) selectively speeds up a reaction without itself being consumed. (You learned about catalysts in Concept 5.4.)

An enzyme catalyzes a reaction by lowering the E_A barrier (**Figure 6.14**), enabling the reactant molecules to absorb enough energy to reach the transition state even at moderate temperatures, as we’ll discuss shortly. It is crucial to note that *an enzyme cannot change the ΔG for a reaction; it cannot make an endergonic reaction exergonic*. Enzymes can only hasten reactions

▼ **Figure 6.14 The effect of an enzyme on activation energy.** Without affecting the free-energy change (ΔG) for a reaction, an enzyme speeds the reaction by reducing its activation energy (E_A).



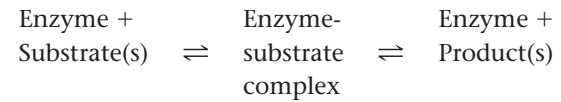
Animation: How Enzymes Work

that would eventually occur anyway, but this enables the cell to have a dynamic metabolism, routing chemicals smoothly through metabolic pathways. Also, enzymes are very specific for the reactions they catalyze, so they determine which chemical processes will be going on in the cell at any given time.

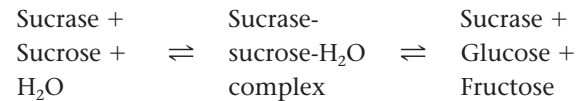
Substrate Specificity of Enzymes

The reactant an enzyme acts on is referred to as the enzyme's **substrate**. The enzyme binds to its substrate (or substrates, when there are two or more reactants), forming an **enzyme-substrate complex**. While enzyme and substrate are joined, the catalytic action of the enzyme converts the

substrate to the product (or products) of the reaction. The overall process can be summarized as follows:



Most enzyme names end in *-ase*. For example, the enzyme **sucrase** catalyzes the hydrolysis of the disaccharide **sucrose** into its two monosaccharides, **glucose** and **fructose** (see the diagram at the beginning of Concept 6.4):



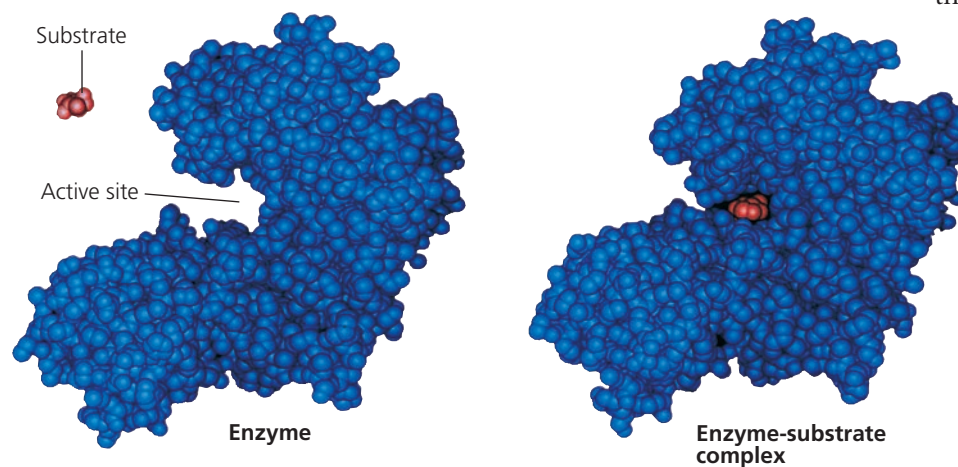
The reaction catalyzed by each enzyme is very specific; an enzyme can recognize its specific substrate even among closely related compounds. For instance, sucrase will act only on sucrose and will not bind to other disaccharides, such as maltose. What accounts for this molecular recognition? Recall that most enzymes are proteins, and proteins are macromolecules with unique three-dimensional configurations. The specificity of an enzyme results from its shape, which is a consequence of its amino acid sequence.

Only a restricted region of the enzyme molecule actually binds to the substrate. This region, called the **active site**, is typically a pocket or groove on the surface of the enzyme where catalysis occurs (Figure 6.15a; see also Figure 5.16). Usually, the active site is formed by only a few of the enzyme's amino acids, with the rest of the protein molecule providing a framework that determines the shape of the active site. The specificity of an enzyme is attributed to a complementary fit between the shape of its active site and the shape of the substrate.

An enzyme is not a stiff structure locked into a given shape. In fact, recent work by biochemists has shown that enzymes (and other proteins) seem to

“dance” between subtly different shapes in a dynamic equilibrium, with slight differences in free energy for each “pose.” The shape that best fits the substrate isn't necessarily the one with the lowest energy, but during the very short time the enzyme takes on this shape, its active site can bind to the substrate. The active site itself is also not a rigid receptacle for the substrate. As the substrate enters the active site, the enzyme changes shape slightly due to interactions between the substrate's chemical groups and chemical groups on the side chains of the amino acids that form the active site. This shape change makes the active site fit even more snugly around the substrate (Figure 6.15b).

▼ **Figure 6.15 Induced fit between an enzyme and its substrate.**



(a) In this space-filling model of the enzyme hexokinase (blue), the active site forms a groove on the surface. The enzyme's substrate is glucose (red).

(b) When the substrate enters the active site, it forms weak bonds with the enzyme, inducing a change in the shape of the protein. This change allows additional weak bonds to form, causing the active site to enfold the substrate and hold it in place.

The tightening of the binding after initial contact—called **induced fit**—is like a clasping handshake. Induced fit brings chemical groups of the active site into positions that enhance their ability to catalyze the chemical reaction.

Catalysis in the Enzyme's Active Site

In most enzymatic reactions, the substrate is held in the active site by so-called weak interactions, such as hydrogen bonds and ionic bonds. The R groups of a few of the amino acids that make up the active site catalyze the conversion of substrate to product, and the product departs from the active site. The enzyme is then free to take another substrate molecule into its active site. The entire cycle happens so fast that a single enzyme molecule typically acts on about 1,000 substrate molecules per second, and some enzymes are even faster. Enzymes, like other catalysts, emerge from the reaction in their original form. Therefore, very small amounts of enzyme can have a huge metabolic impact by functioning over and over again in catalytic cycles. **Figure 6.16** shows a catalytic cycle involving two substrates and two products.

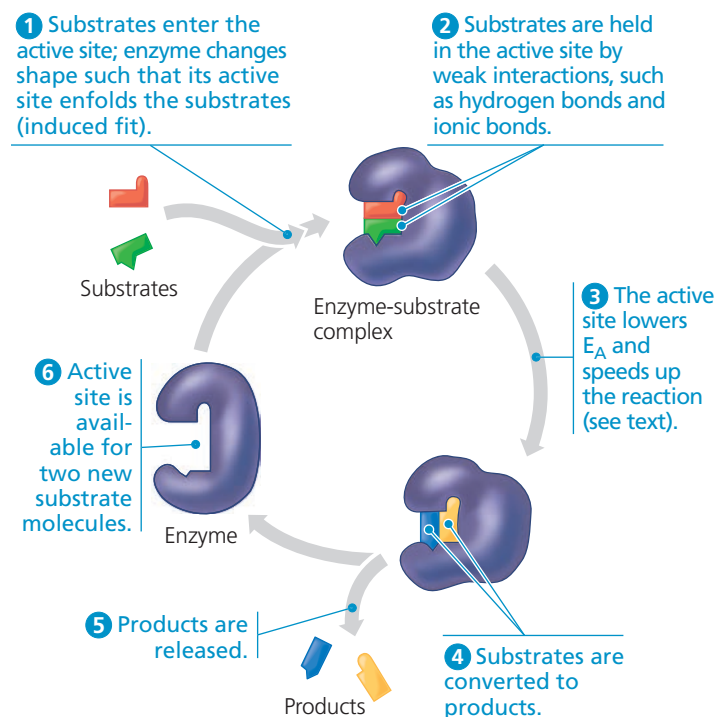
Most metabolic reactions are reversible, and an enzyme can catalyze either the forward or the reverse reaction, depending on which direction has a negative ΔG . This in turn depends mainly on the relative concentrations of reactants and products. The net effect is always in the direction of equilibrium.

Enzymes use a variety of mechanisms that lower activation energy and speed up a reaction (see Figure 6.16, step 3):

- When there are two or more reactants, the active site provides a template on which the substrates can come together in the proper orientation for a reaction to occur between them.
- As the active site of an enzyme clutches the bound substrates, the enzyme may stretch the substrate molecules toward their transition state form, stressing and bending critical chemical bonds to be broken during the reaction. Because E_A is proportional to the difficulty of breaking the bonds, distorting the substrate helps it approach the transition state and reduces the amount of free energy that must be absorbed to achieve that state.
- The active site may also provide a microenvironment that is more conducive to a particular type of reaction than the solution itself would be without the enzyme. For example, if the active site has amino acids with acidic R groups, the active site may be a pocket of low pH in an otherwise neutral cell. In such cases, an acidic amino acid may facilitate H^+ transfer to the substrate as a key step in catalyzing the reaction.
- Amino acids in the active site directly participate in the chemical reaction. Sometimes this process even involves brief covalent bonding between the substrate and the

▼ Figure 6.16 The active site and catalytic cycle of an enzyme.

An enzyme can convert one or more reactant molecules to one or more product molecules. The enzyme shown here converts two substrate molecules to two product molecules.



DRAW IT ▶ The enzyme-substrate complex passes through a transition state (see Figure 6.13). Label the part of the cycle where the transition state occurs.

MB Animation: Enzymes: Steps in a Reaction

side chain of an amino acid of the enzyme. Subsequent steps of the reaction restore the side chains to their original states so that the active site is the same after the reaction as it was before.

The rate at which a particular amount of enzyme converts substrate to product is partly a function of the initial concentration of the substrate: The more substrate molecules that are available, the more frequently they access the active sites of the enzyme molecules. However, there is a limit to how fast the reaction can be pushed by adding more substrate to a fixed concentration of enzyme. At some point, the concentration of substrate will be high enough that all enzyme molecules will have their active sites engaged. As soon as the product exits an active site, another substrate molecule enters. At this substrate concentration, the enzyme is said to be *saturated*, and the rate of the reaction is determined by the speed at which the active site converts substrate to product. When an enzyme population is saturated, the only way to increase the rate of product formation is to add more enzyme. Cells often increase the rate of a reaction by producing more enzyme molecules. You can graph the overall progress of an enzymatic reaction in the **Scientific Skills Exercise**.

SCIENTIFIC SKILLS EXERCISE

Making a Line Graph and Calculating a Slope

Does the Rate of Glucose 6-Phosphatase Activity Change over Time in Isolated Liver Cells? Glucose 6-phosphatase, which is found in mammalian liver cells, is a key enzyme in control of blood glucose levels. The enzyme catalyzes the breakdown of glucose 6-phosphate into glucose and inorganic phosphate (P_i). These products are transported out of liver cells into the blood, increasing blood glucose levels. In this exercise, you will graph data from a time-course experiment that measured P_i concentration in the buffer outside isolated liver cells, thus indirectly measuring glucose 6-phosphatase activity inside the cells.

How the Experiment Was Done Isolated rat liver cells were placed in a dish with buffer at physiological conditions (pH 7.4, 37°C). Glucose 6-phosphate (the substrate) was added so it could be taken up by cells and broken down by glucose 6-phosphatase. A sample of buffer was removed every 5 minutes and the concentration of P_i that had been transported out of the cells was determined

Data from the Experiment

Time (min)	Concentration of P_i ($\mu\text{mol/mL}$)
0	0
5	10
10	90
15	180
20	270
25	330
30	355
35	355
40	355

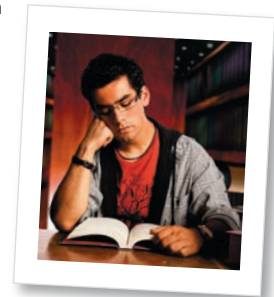
Data from S. R. Commerford et al., Diets enriched in sucrose or fat increase gluconeogenesis and G-6-Pase but not basal glucose production in rats, *American Journal of Physiology - Endocrinology and Metabolism* 283:E545–E555 (2002).

INTERPRET THE DATA

1. To see patterns in the data from a time-course experiment like this, it is helpful to graph the data. First, determine which set of

data goes on each axis. (a) What did the researchers intentionally vary in the experiment? This is the independent variable, which goes on the x-axis. (b) What are the units (abbreviated) for the independent variable? Explain in words what the abbreviation stands for. (c) What was measured by the researchers? This is the dependent variable, which goes on the y-axis. (d) What does the units abbreviation stand for? Label each axis, including the units.

2. Next, you'll want to mark off the axes with just enough evenly spaced tick marks to accommodate the full set of data. Determine the range of data values for each axis. (a) What is the largest value to go on the x-axis? What is a reasonable spacing for the tick marks, and what should be the highest one? (b) What is the largest value to go on the y-axis? What is a reasonable spacing for the tick marks, and what should be the highest one?
3. Plot the data points on your graph. Match each x-value with its partner y-value and place a point on the graph at that coordinate. Draw a line that connects the points. (For additional information about graphs, see the Scientific Skills Review in Appendix F.)
4. Examine your graph and look for patterns in the data. (a) Does the concentration of P_i increase evenly through the course of the experiment? To answer this question, describe the pattern you see in the graph. (b) What part of the graph shows the highest rate of enzyme activity? Consider that the rate of enzyme activity is related to the slope of the line, $\Delta y/\Delta x$ (the "rise" over the "run"), in $\mu\text{mol}/(\text{mL} \cdot \text{min})$, with the steepest slope indicating the highest rate of enzyme activity. Calculate the rate of enzyme activity (slope) where the graph is steepest. (c) Can you think of a biological explanation for the pattern you see?
5. If your blood sugar level is low from skipping lunch, what reaction (discussed in this exercise) will occur in your liver cells? Write out the reaction and put the name of the enzyme over the reaction arrow. How will this reaction affect your blood sugar level?



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Effects of Local Conditions on Enzyme Activity

The activity of an enzyme—how efficiently the enzyme functions—is affected by general environmental factors, such as temperature and pH. It can also be affected by chemicals that specifically influence that enzyme. In fact, researchers have learned much about enzyme function by employing such chemicals.

Effects of Temperature and pH

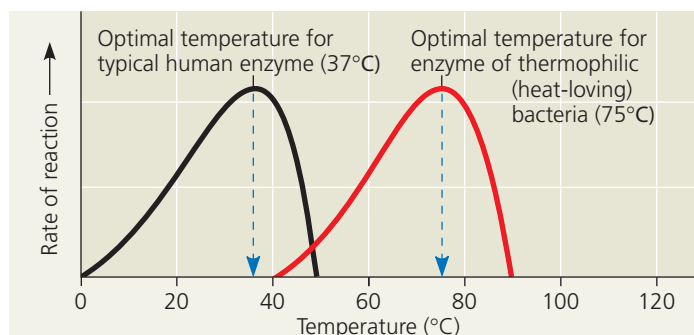
Recall from Figure 5.20 that the three-dimensional structures of proteins are sensitive to their environment. As a consequence, each enzyme works better under some conditions than under other conditions, because these *optimal conditions* favor the most active shape for the enzyme.

Temperature and pH are environmental factors important in the activity of an enzyme. Up to a point, the rate of an enzymatic reaction increases with increasing temperature, partly because substrates collide with active sites more frequently when the molecules move rapidly. Above that temperature, however, the speed of the enzymatic reaction drops sharply. The thermal agitation of the enzyme molecule disrupts the hydrogen bonds, ionic bonds, and other weak interactions that stabilize the active shape of the enzyme, and the protein molecule eventually denatures. Each enzyme has an optimal temperature at which its reaction rate is greatest. Without denaturing the enzyme, this temperature allows the greatest number of molecular collisions and the fastest conversion of the reactants to product molecules. Most human enzymes have optimal temperatures of about 35–40°C

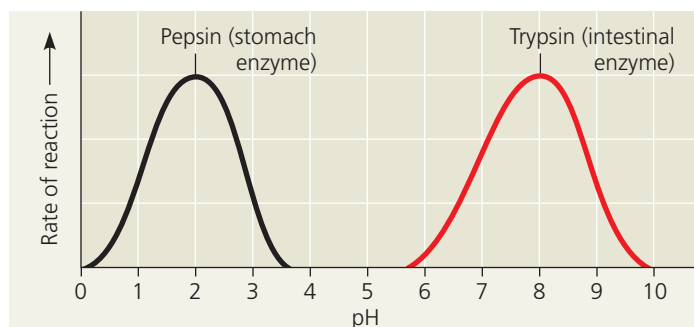
(close to human body temperature). The thermophilic bacteria that live in hot springs contain enzymes with optimal temperatures of 70°C or higher (**Figure 6.17a**).

Just as each enzyme has an optimal temperature, it also has a pH at which it is most active. The optimal pH values for most

▼ **Figure 6.17 Environmental factors affecting enzyme activity.** Each enzyme has an optimal (a) temperature and (b) pH that favor the most active shape of the protein molecule.



(a) The photo shows thermophilic cyanobacteria (green) thriving in the hot water of a Nevada geyser. The graph compares the optimal temperatures for an enzyme from the thermophilic bacterium *Thermus oshimai* (75°C) and human enzymes (body temperature, 37°C).



(b) This graph shows the rate of reaction for two digestive enzymes over a range of pH values.

INTERPRET THE DATA ► Looking at the graph in (b), what is the optimal pH for pepsin activity? Explain why natural selection might have resulted in the optimal pH for pepsin, a stomach enzyme (see Figure 3.11). What is the optimal pH for trypsin?

enzymes fall in the range of pH 6–8, but there are exceptions. For example, pepsin, a digestive enzyme in the human stomach, works best at a very low pH. Such an acidic environment denatures most enzymes, but pepsin is adapted to maintain its functional three-dimensional structure in the acidic environment of the stomach. In contrast, trypsin, a digestive enzyme residing in the more alkaline environment of the human intestine would be denatured in the stomach (**Figure 6.17b**).

Cofactors

Many enzymes require nonprotein helpers for catalytic activity, often for chemical processes like electron transfers that cannot easily be carried out by the amino acids in proteins. These adjuncts, called **cofactors**, may be bound tightly to the enzyme as permanent residents, or they may bind loosely and reversibly along with the substrate. The cofactors of some enzymes are inorganic, such as the metal atoms zinc, iron, and copper in ionic form. If the cofactor is an organic molecule, it is referred to, more specifically, as a **coenzyme**. Most vitamins are important in nutrition because they act as coenzymes or raw materials from which coenzymes are made.

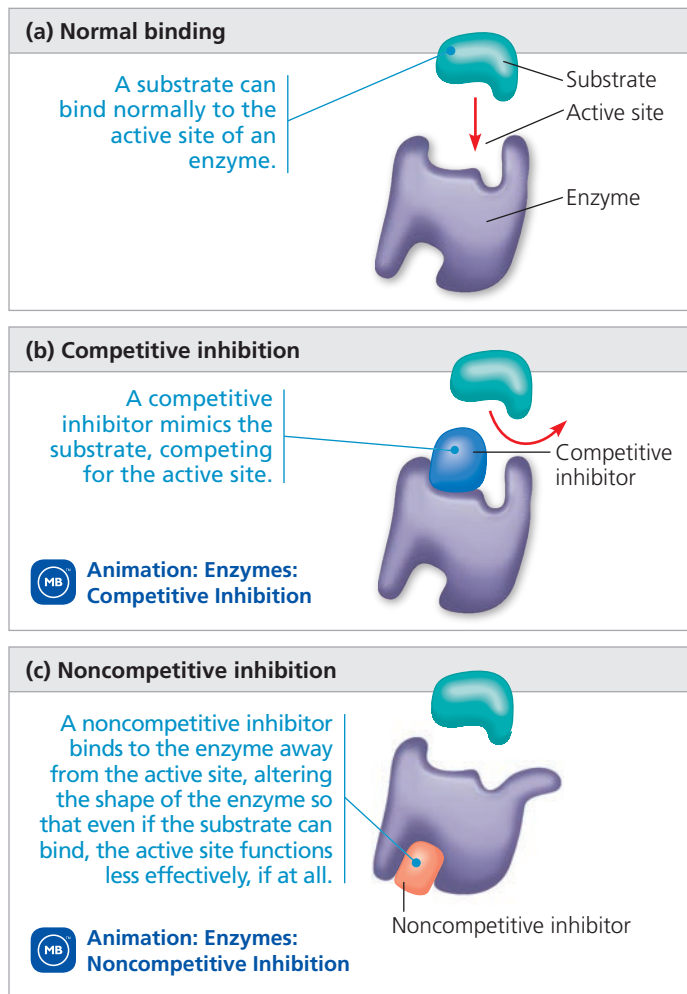
Enzyme Inhibitors

Certain chemicals selectively inhibit the action of specific enzymes. Sometimes the inhibitor attaches to the enzyme by covalent bonds, in which case the inhibition is usually irreversible. Many enzyme inhibitors, however, bind to the enzyme by weak interactions, and when this occurs the inhibition is reversible. Some reversible inhibitors resemble the normal substrate molecule and compete for admission into the active site (**Figure 6.18a** and **b**). These mimics, called **competitive inhibitors**, reduce the productivity of enzymes by blocking substrates from entering active sites. This kind of inhibition can be overcome by increasing the concentration of substrate so that as active sites become available, more substrate molecules than inhibitor molecules are around to gain entry to the sites.

In contrast, **noncompetitive inhibitors** do not directly compete with the substrate to bind to the enzyme at the active site (**Figure 6.18c**). Instead, they impede enzymatic reactions by binding to another part of the enzyme. This interaction causes the enzyme molecule to change its shape in such a way that the active site becomes much less effective at catalyzing the conversion of substrate to product.

Toxins and poisons are often irreversible enzyme inhibitors. An example is sarin, a nerve gas. In the mid-1990s, terrorists released sarin in the Tokyo subway, killing several people and injuring many others. This small molecule binds covalently to the R group on the amino acid serine, which is found in the active site of acetylcholinesterase, an enzyme important in the nervous system. Other examples include the pesticides DDT and parathion, inhibitors of key enzymes in the nervous system. Finally, many antibiotics are inhibitors of specific

▼ **Figure 6.18** Inhibition of enzyme activity.



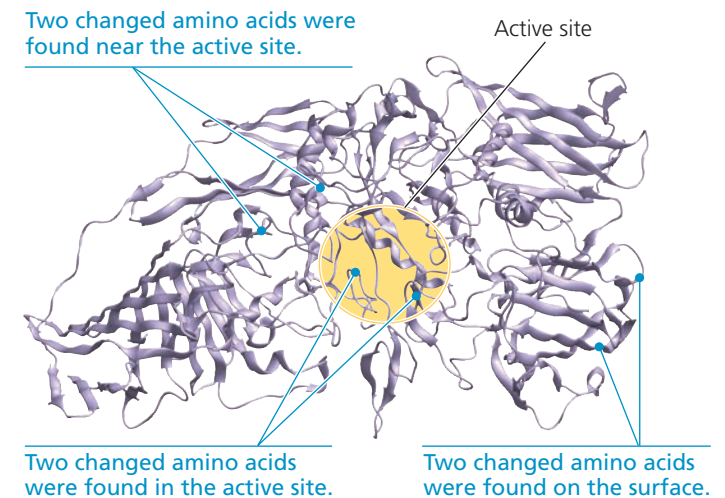
enzymes in bacteria. For instance, penicillin blocks the active site of an enzyme that many bacteria use to make cell walls.

Citing enzyme inhibitors that are metabolic poisons may give the impression that enzyme inhibition is generally abnormal and harmful. In fact, molecules naturally present in the cell often regulate enzyme activity by acting as inhibitors. Such regulation—selective inhibition—is essential to the control of cellular metabolism, as we will discuss in Concept 6.5.

The Evolution of Enzymes

EVOLUTION Thus far, biochemists have identified more than 4,000 different enzymes in various species, most likely a very small fraction of all enzymes. How did this grand profusion of enzymes arise? Recall that most enzymes are proteins, and proteins are encoded by genes. A permanent change in a gene, known as a *mutation*, can result in a protein with one or more changed amino acids. In the case of an enzyme, if the changed amino acids are in the active site or some other crucial region, the altered enzyme might have a novel activity or might bind to a different substrate. Under environmental conditions where the new function benefits the organism, natural selection would tend to favor the mutated form of the gene, causing it to persist in the population.

▼ **Figure 6.19** Mimicking evolution of an enzyme with a new function. Researchers tested whether the function of an enzyme called β -galactosidase, which breaks down the sugar lactose, could change over time in populations of the bacterium *Escherichia coli*. After seven rounds of mutation and selection in the lab, β -galactosidase evolved into an enzyme specialized for breaking down a sugar other than lactose. This ribbon model shows one subunit of the altered enzyme; six amino acids were different.



This simplified model is generally accepted as the main way in which the multitude of different enzymes arose over the past few billion years of life's history. Data supporting this model have been collected by researchers using a lab procedure that mimics evolution in natural populations (**Figure 6.19**).

CONCEPT CHECK 6.4

1. Many spontaneous reactions occur very slowly. Why don't all spontaneous reactions occur instantly?
2. Why do enzymes act only on very specific substrates?
3. **WHAT IF?** β -galactosidase breaks down the sugar lactose. How would you determine if it can break down maltose due to some mutation in its gene?
4. **DRAW IT** An organelle called a lysosome has an internal pH of around 4.5. Using Figure 6.17b as a guide, draw a graph showing what you would predict for the rate of reaction for a lysosomal enzyme. Label its optimal pH, assuming its optimal pH matches its environment.

For suggested answers, see Appendix A.

CONCEPT 6.5

Regulation of enzyme activity helps control metabolism

Chemical chaos would result if all of a cell's metabolic pathways were operating simultaneously. Intrinsic to life's processes is a cell's ability to tightly regulate its metabolic pathways by controlling when and where its various enzymes are active. It does this either by switching on and off the genes that encode specific enzymes or by regulating the activity of enzymes once they are made.

Allosteric Regulation of Enzymes

In many cases, the molecules that naturally regulate enzyme activity in a cell behave something like reversible noncompetitive inhibitors (see Figure 6.18c): These regulatory molecules change an enzyme's shape and the functioning of its active site by binding to a site elsewhere on the molecule, via noncovalent interactions. **Allosteric regulation** is the term used to describe any case in which a protein's function at one site is affected by the binding of a regulatory molecule to a separate site. It may result in either inhibition or stimulation of an enzyme's activity.

Allosteric Activation and Inhibition

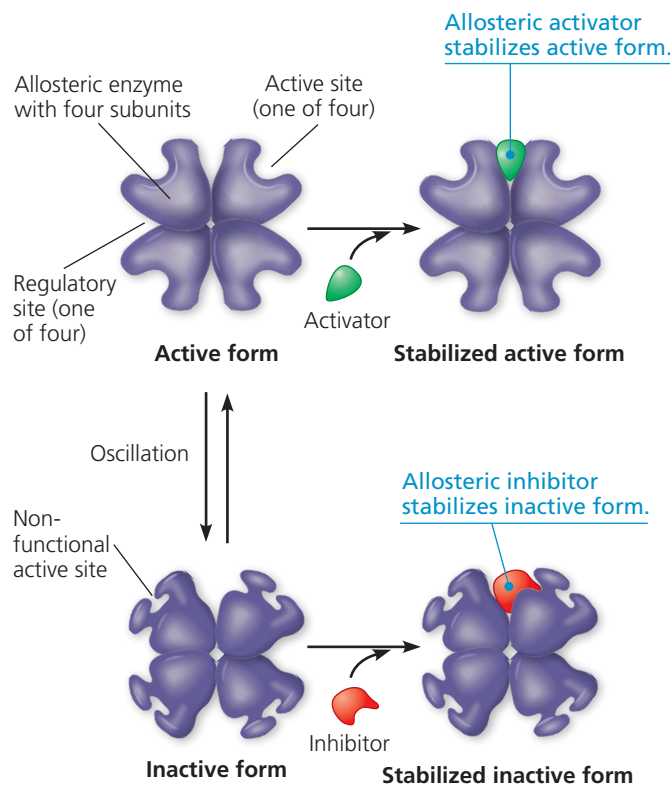
Most enzymes known to be allosterically regulated are constructed from two or more subunits, each composed of a polypeptide chain with its own active site. The entire complex oscillates between two different shapes, one catalytically active and the other inactive (**Figure 6.20a**). In the simplest kind of allosteric regulation, an activating or inhibiting regulatory molecule binds to a regulatory site (sometimes called an allosteric site), often located where subunits join. The binding of an *activator* to a regulatory site stabilizes the shape that has functional active sites, whereas the binding of an *inhibitor* stabilizes the inactive form of the enzyme. The subunits of an allosteric enzyme fit together in such a way that a shape change in one subunit is transmitted to all others. Through this interaction of subunits, a single activator or inhibitor molecule that binds to one regulatory site will affect the active sites of all subunits.

Fluctuating concentrations of regulators can cause a sophisticated pattern of response in the activity of cellular enzymes. The products of ATP hydrolysis (ADP and P_i), for example, play a complex role in balancing the flow of traffic between anabolic and catabolic pathways by their effects on key enzymes. ATP binds to several catabolic enzymes allosterically, lowering their affinity for substrate and thus inhibiting their activity. ADP, however, functions as an activator of the same enzymes. This is logical because catabolism functions in regenerating ATP. If ATP production lags behind its use, ADP accumulates and activates the enzymes that speed up catabolism, producing more ATP. If the supply of ATP exceeds demand, then catabolism slows down as ATP molecules accumulate and bind to the same enzymes, inhibiting them. (You'll see specific examples of this type of regulation when you learn about cellular respiration in Chapter 10; see, for example, Figure 10.20.) ATP, ADP, and other related molecules also affect key enzymes in anabolic pathways. In this way, allosteric enzymes control the rates of important reactions in both sorts of metabolic pathways.

In another kind of allosteric activation, a *substrate* molecule binding to one active site in a multisubunit enzyme triggers a shape change in all the subunits, thereby increasing catalytic activity at the other active sites (**Figure 6.20b**). Called **cooperativity**, this mechanism amplifies the response of enzymes to substrates: One substrate molecule

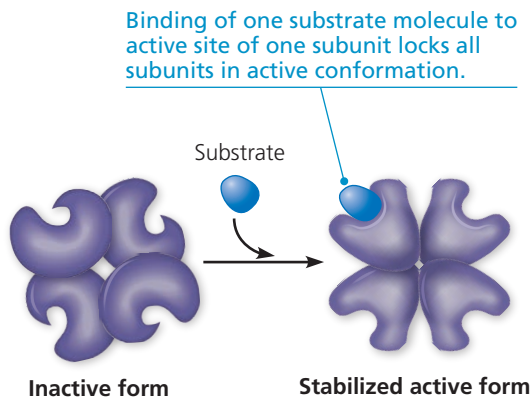
▼ **Figure 6.20** Allosteric regulation of enzyme activity.

(a) Allosteric activators and inhibitors



At low concentrations, activators and inhibitors dissociate from the enzyme. The enzyme can then oscillate again.

(b) Cooperativity: another type of allosteric activation



The inactive form shown on the left oscillates with the active form when the active form is not stabilized by substrate.

primes an enzyme to act on additional substrate molecules more readily. Cooperativity is considered allosteric regulation because, even though substrate is binding to an active site, its binding affects catalysis in another active site.

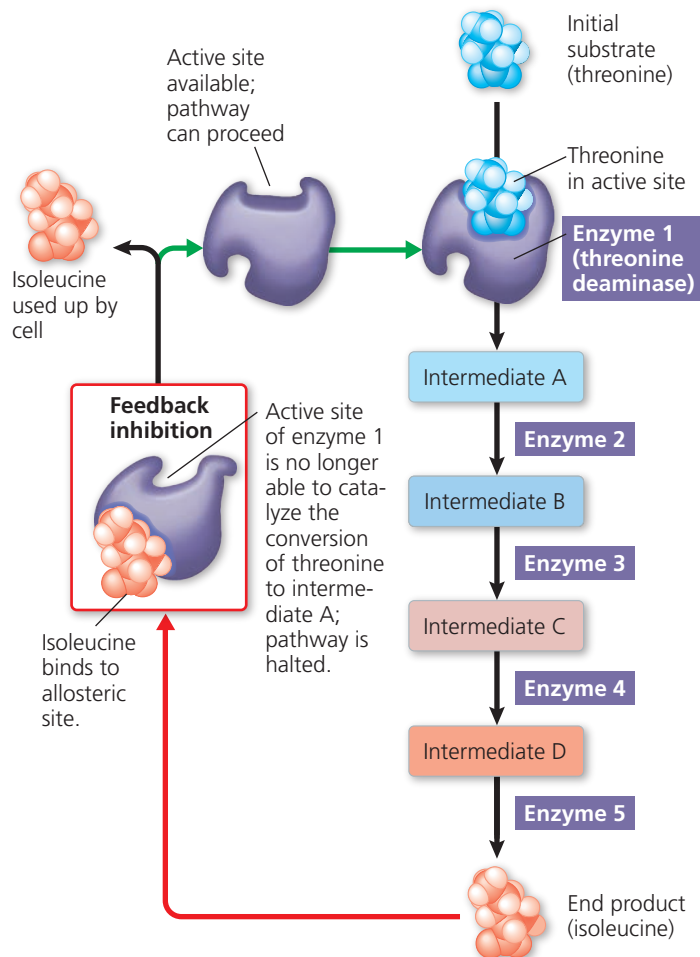
Although hemoglobin is not an enzyme (it carries O_2 rather than catalyzing a reaction), classic studies of hemoglobin have elucidated the principle of cooperativity. Hemoglobin is made up of four subunits, each with an oxygen-binding site (see Figure 5.18). The binding of an oxygen molecule to one

binding site increases the affinity for oxygen of the remaining binding sites. Thus, where oxygen is at high levels, such as in the lungs or gills, hemoglobin's affinity for oxygen increases as more binding sites are filled. In oxygen-deprived tissues, however, the release of each oxygen molecule decreases the oxygen affinity of the other binding sites, resulting in the release of oxygen where it is most needed. Cooperativity works similarly in multisubunit enzymes that have been studied.

Feedback Inhibition

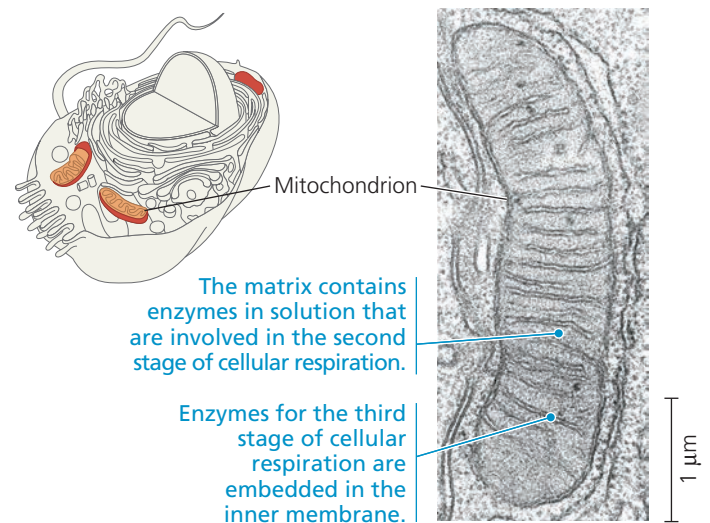
Earlier, we discussed the allosteric inhibition of an enzyme in an ATP-generating pathway by ATP itself. This is a common mode of metabolic control, called **feedback inhibition**, in which a metabolic pathway is halted by the inhibitory binding of its end product to an enzyme that acts early in the pathway. **Figure 6.21** shows an example of feedback inhibition operating on an anabolic pathway. Some cells use this five-step pathway to synthesize the amino acid isoleucine from threonine, another amino acid. As isoleucine accumulates, it slows down its own synthesis by allosterically inhibiting the enzyme for the first step of the pathway. Feedback inhibition thereby prevents the cell from making more isoleucine than is necessary and thus wasting chemical resources.

▼ **Figure 6.21** Feedback inhibition in isoleucine synthesis.



▼ **Figure 6.22** Organelles and structural order in metabolism.

Organelles such as the mitochondrion contain enzymes that carry out specific functions, in this case the second and third stages of cellular respiration.



Localization of Enzymes Within the Cell

The cell is not just a bag of chemicals with thousands of different kinds of enzymes and substrates in a random mix. The cell is compartmentalized, and cellular structures help bring order to metabolic pathways. In some cases, a team of enzymes for several steps of a metabolic pathway are assembled into a multienzyme complex. The arrangement facilitates the sequence of reactions, with the product from the first enzyme becoming the substrate for an adjacent enzyme in the complex, and so on, until the end product is released. Some enzymes and enzyme complexes have fixed locations within the cell and act as structural components of particular membranes. Others are in solution within particular membrane-enclosed eukaryotic organelles, each with its own internal chemical environment. For example, in eukaryotic cells, the enzymes for the second and third stages of cellular respiration reside in specific locations within mitochondria (**Figure 6.22**).

In this chapter, you have learned about the laws of thermodynamics that govern metabolism, the intersecting set of chemical pathways characteristic of life. We have explored the bioenergetics of breaking down and building up biological molecules. To continue the theme of bioenergetics, we will next examine the structure of eukaryotic cells in detail, focusing on the various organelles in which specific chemical reactions take place.

CONCEPT CHECK 6.5

1. How do an activator and an inhibitor have different effects on an allosterically regulated enzyme?
2. **WHAT IF? >** Regulation of isoleucine synthesis is an example of feedback inhibition of an anabolic pathway. With that in mind, explain how ATP might be involved in feedback inhibition of a catabolic pathway.

For suggested answers, see Appendix A.

6 Chapter Review



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SUMMARY OF KEY CONCEPTS

CONCEPT 6.1

An organism's metabolism transforms matter and energy, subject to the laws of thermodynamics (pp. 142–145)



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax

- **Metabolism** is the collection of chemical reactions that occur in an organism. Enzymes catalyze reactions in intersecting **metabolic pathways**, which may be **catabolic** (breaking down molecules, releasing energy) or **anabolic** (building molecules, consuming energy). **Bioenergetics** is the study of the flow of energy through living organisms.
- **Energy** is the capacity to cause change; some forms of energy do work by moving matter. **Kinetic energy** is associated with motion and includes **thermal energy** associated with random motion of atoms or molecules. **Heat** is thermal energy in transfer from one object to another. **Potential energy** is related to the location or structure of matter and includes **chemical energy** possessed by a molecule due to its structure.
- **The first law of thermodynamics**, conservation of energy, states that energy cannot be created or destroyed, only transferred or transformed. The **second law of thermodynamics** states that **spontaneous processes**, those requiring no outside input of energy, increase the **entropy** (molecular disorder) of the universe.

? Explain how the highly ordered structure of a cell does not conflict with the second law of thermodynamics.

CONCEPT 6.2

The free-energy change of a reaction tells us whether or not the reaction occurs spontaneously (pp. 145–148)

- A living system's **free energy** is energy that can do work under cellular conditions. The change in free energy (ΔG) during a biological process is related directly to enthalpy change (ΔH) and to the change in entropy (ΔS): $\Delta G = \Delta H - T\Delta S$. Organisms live at the expense of free energy. A spontaneous process occurs with no energy input; during such a process, free energy decreases and the stability of a system increases. At maximum stability, the system is at equilibrium and can do no work.
- In an **exergonic** (spontaneous) chemical reaction, the products have less free energy than the reactants ($-\Delta G$). **Endergonic** (nonspontaneous) reactions require an input of energy ($+\Delta G$). The addition of starting materials and the removal of end products prevent metabolism from reaching equilibrium.

? Explain the meaning of each component in the equation for the change in free energy of a spontaneous chemical reaction. Why are spontaneous reactions important in the metabolism of a cell?

CONCEPT 6.3

ATP powers cellular work by coupling exergonic reactions to endergonic reactions (pp. 148–151)

- **ATP** is the cell's energy shuttle. Hydrolysis of its terminal phosphate yields ADP and P_i and releases free energy.
- Through **energy coupling**, the exergonic process of ATP hydrolysis drives endergonic reactions by transfer of a phosphate group to specific reactants, forming a **phosphorylated intermediate**

that is more reactive. ATP hydrolysis (sometimes with protein phosphorylation) also causes changes in the shape and binding affinities of transport and motor proteins.

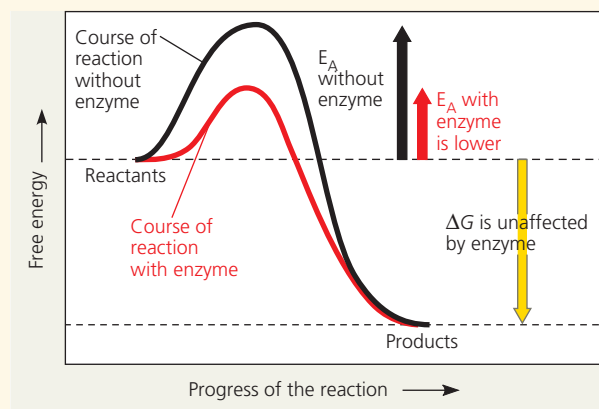
- Catabolic pathways drive regeneration of ATP from ADP + P_i .

? Describe the ATP cycle: How is ATP used and regenerated in a cell?

CONCEPT 6.4

Enzymes speed up metabolic reactions by lowering energy barriers (pp. 151–157)

- In a chemical reaction, the energy necessary to break the bonds of the reactants is the **activation energy**, E_A .
- **Enzymes** lower the E_A barrier:



- Each enzyme has a unique **active site** that binds one or more **substrate(s)**, the reactants on which it acts. It then changes shape, binding the substrate(s) more tightly (**induced fit**).
- The active site can lower an E_A barrier by orienting substrates correctly, straining their bonds, providing a favorable micro-environment, or even covalently bonding with the substrate.
- Each enzyme has an optimal temperature and pH. Inhibitors reduce enzyme function. A **competitive inhibitor** binds to the active site, whereas a **noncompetitive inhibitor** binds to a different site on the enzyme.
- Natural selection, acting on organisms with variant enzymes, is responsible for the diversity of enzymes found in organisms.

? How do both activation energy barriers and enzymes help maintain the structural and metabolic order of life?

CONCEPT 6.5

Regulation of enzyme activity helps control metabolism (pp. 157–159)

- Many enzymes are subject to **allosteric regulation**: Regulatory molecules, either activators or inhibitors, bind to specific regulatory sites, affecting the shape and function of the enzyme. In **cooperativity**, binding of one substrate molecule can stimulate binding or activity at other active sites. In **feedback inhibition**, the end product of a metabolic pathway allosterically inhibits the enzyme for a previous step in the pathway.
- Some enzymes are grouped into complexes, some are incorporated into membranes, and some are contained inside organelles, increasing the efficiency of metabolic processes.

? What roles do allosteric regulation and feedback inhibition play in the metabolism of a cell?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. **EVOLUTION CONNECTION** Some people argue that biochemical pathways are too complex to have evolved because all intermediate steps in a given pathway must be present to produce the final product. Critique this argument. How could you use the diversity of metabolic pathways that produce the same or similar products to support your case?
9. **SCIENTIFIC INQUIRY • DRAW IT** A researcher has developed an assay to measure the activity of an important enzyme present in pancreatic cells growing in culture. She adds the enzyme's substrate to a dish of cells and then measures the appearance of reaction products. The results are graphed as the amount of product on the y -axis versus time on the x -axis. The researcher notes four sections of the graph. For a short period of time, no products appear (section A). Then (section B) the reaction rate is quite high (the slope of the line is steep). Next, the reaction gradually slows down (section C). Finally, the graph line becomes flat (section D). Draw and label the graph, and propose a model to explain the molecular events occurring at each stage of this reaction profile.
10. **WRITE ABOUT A THEME: ENERGY AND MATTER** The laws of thermodynamics explain the relationship between energy and matter. In a short essay (100–150 words), describe how the first and second laws of thermodynamics form the basis of energy transformations in organisms.



PRACTICE
TEST
goo.gl/iAsVgI

11. SYNTHESIZE YOUR KNOWLEDGE



Explain what is happening in this photo in terms of kinetic energy and potential energy. Include the energy conversions that occur when the penguins eat fish and climb back up on the glacier. Describe the role of ATP and enzymes in the underlying molecular processes, including what happens to the free energy of some of the molecules involved.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

UNIT 2

CELL BIOLOGY

Elba Serrano was born in Old San Juan, Puerto Rico. Because her father was a career sergeant in the U.S. military, she had a nomadic childhood, tempered by her family's strong Hispanic culture. She majored in physics at the University of Rochester, one of two women among 80 majors. She then earned a Ph.D. from Stanford University, studying the permeability of nerve cells to ions, and was a postdoctoral fellow at UCLA. There, she started working on the auditory system, as a way of understanding how cells allow an organism to respond to changes in the environment. Since 1992, Dr. Serrano has been a faculty member at New Mexico State University, where she has made many discoveries about the development of the auditory system. Dr. Serrano is also an outspoken advocate and mentor for women and underrepresented groups in science.



An Interview with Elba Serrano

How did you get interested in science, and in biology in particular?

When you're a military kid, you're asked to pick out what you want for Christmas from catalogs, and I remember always gravitating to the telescope and the chemistry set. To this day, I love math—I solve math problems for fun. In college I studied in a medical school library, and I'd take breaks from studying physics by reading about biology. I became very interested in biochemistry, and I said, "It looks like there is a field called biophysics—I think I'll go to grad school for that." I ended up at Stanford, and I really struggled—I was pretty much ready to drop out at the end of the first year.

Then I was assigned a book called *Nerve, Muscle, and Synapse*, by Bernard Katz. I started reading this book at 8 AM and didn't stop—I didn't eat lunch. I finished it by the end of the day and knew "this is what I want to do." It was basically membrane channel biophysics, and there were equations! I thought, "This is the science I recognize."

What was your dissertation research?

I worked on neurons, and I focused on the flow of ions like potassium through the cell membrane. Membranes are interesting because they both define a cell—they're barriers—and at the same time they are a conduit. Membranes are like borders—very dynamic places where a lot of things happen. Within the membrane there are special proteins called ion channels, a kind of gatekeeper that permits the passage of ions. Neurons are the basic cells of the brain. The way the brain does its job is through the coordinated work of many neurons, and these cells are similar in some ways but also extremely diverse. So my dissertation question explored the diversity of neuron cell membranes: How are the proteins on different neurons different?

How did you become interested in the auditory system, and what questions are you asking?

I always want to use the concepts of physics to understand biological systems. Biophysicists love the inner ear: it has sensory surfaces with beautiful cells called mechanosensory hair cells (see photo). These are similar but diverse in their function; the ear's capacity to pick up all sound frequencies is because all the little hair cells are slightly different from each other. The mechanosensory hair cells we work on in my lab are marvelous, beautiful, very special cells. We study the structure and positioning of these cells, and at the same time we ask genetics questions. We are interested in identifying the genes for the ion channel proteins that are important in the electrical response of these cells—how are they organized in the genome, how are they turned on and off? The ear is a powerful system that has both redundancy—many similar cells—and diversity—each cell is unique.

What is your advice to an undergraduate who is considering a career in biology?

First, don't limit yourself in your possibilities for your future. Next, get a variety of experiences—part of life's journey is about finding out what gives us satisfaction. If you're choosing a career in biology, there are many ways you can be a biologist. You can work in labs or in field settings, among others. Finally, understanding biology today requires understanding math, physics, and chemistry, so it is important to invest in those areas. Even if you feel you will not be able to excel in them, do not let that deter you, because everything we do in science can be learned—it just requires dedication.

"Membranes are like borders—very dynamic places where a lot of things happen."

▼ Inner ear "hair" cells with bundles of rod-like protrusions that detect sound waves. Damage to these bundles by noise or aging can lead to deafness or loss of balance. (SEM)



5 μ m

Cell Structure and Function

▲ **Figure 7.1** How do your cells help you learn about biology?

KEY CONCEPTS

- 7.1** Biologists use microscopes and biochemistry to study cells
- 7.2** Eukaryotic cells have internal membranes that compartmentalize their functions
- 7.3** The eukaryotic cell's genetic instructions are housed in the nucleus and carried out by the ribosomes
- 7.4** The endomembrane system regulates protein traffic and performs metabolic functions
- 7.5** Mitochondria and chloroplasts change energy from one form to another
- 7.6** The cytoskeleton is a network of fibers that organizes structures and activities in the cell
- 7.7** Extracellular components and connections between cells help coordinate cellular activities
- 7.8** A cell is greater than the sum of its parts

The Fundamental Units of Life

Cells are as fundamental to the living systems of biology as the atom is to chemistry. Many different types of cells are working for you right now. The contraction of muscle cells moves your eyes as you read this sentence. **Figure 7.1** shows extensions from a nerve cell (orange) making contact with muscle cells (red). The words on the page are translated into signals that nerve cells carry to your brain, where they are passed on to other nerve cells. As you study, your cells make connections between nerve cells that solidify memories and permit learning to occur.

All organisms are made of cells. In the hierarchy of biological organization, the cell is the simplest collection of matter that can be considered a living entity. Indeed, many forms of life exist as single-celled organisms, like the *Paramecium* shown here, a eukaryote that lives in pond water. Larger, more complex organisms, including plants and animals, are multicellular; their bodies are associations of many kinds of specialized cells that could not survive for long on their own. Even when cells are arranged into higher levels of organization, such as tissues and organs, the cell remains the organism's basic unit of structure and function.

All cells are related by their descent from earlier cells. During the long evolutionary history of life on Earth, cells have been modified in many different ways. But although cells can differ substantially from one another, they share common features. In this chapter, we'll first examine the tools and techniques that allow us to understand cells, then tour the cell and become acquainted with its components.



When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

CONCEPT 7.1

Biologists use microscopes and biochemistry to study cells

How can cell biologists investigate the inner workings of a cell, usually too small to be seen by the unaided eye? Before we tour the cell, it will be helpful to learn how cells are studied.

Microscopy

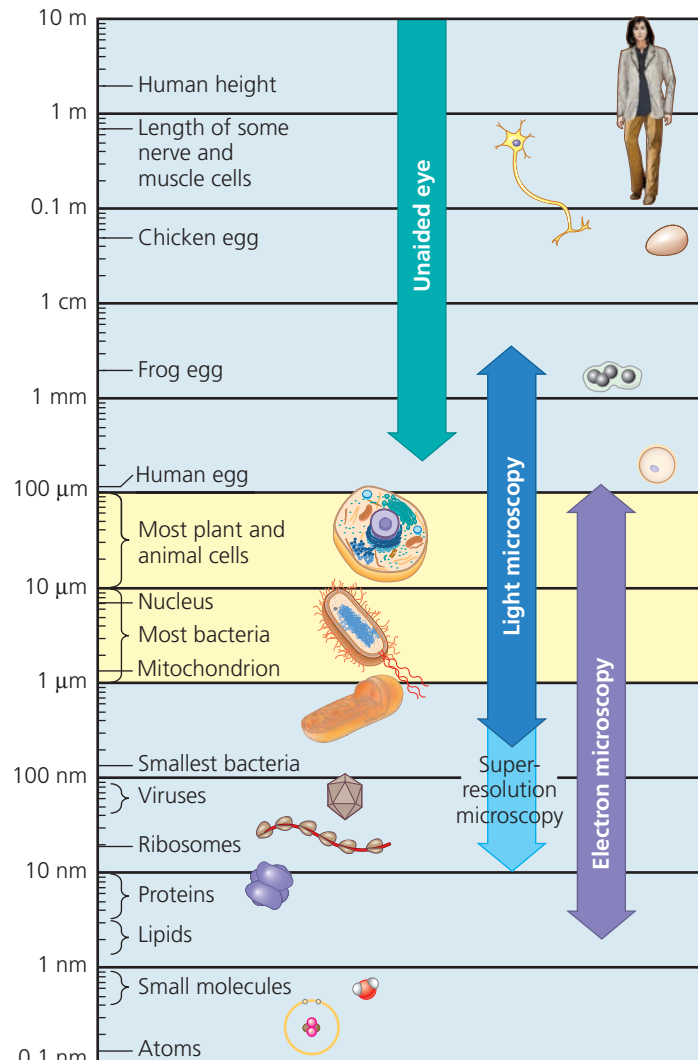
The development of instruments that extend the human senses allowed the discovery and early study of cells. Microscopes were invented in 1590 and further refined during the 1600s. Cell walls were first seen by Robert Hooke in 1665 as he looked through a microscope at dead cells from the bark of an oak tree. But it took the wonderfully crafted lenses of Antoni van Leeuwenhoek to visualize living cells. Imagine Hooke's excitement when he visited van Leeuwenhoek in 1674 and the world of microorganisms—what his host called “very little animalcules”—was revealed to him.

The microscopes first used by Renaissance scientists, as well as the microscopes you are likely to use in the laboratory, are all light microscopes. In a **light microscope (LM)**, visible light is passed through the specimen and then through glass lenses. The lenses refract (bend) the light in such a way that the image of the specimen is magnified as it is projected into the eye or into a camera (see Appendix D).

Three important parameters in microscopy are magnification, resolution, and contrast. *Magnification* is the ratio of an object's image size to its real size. Light microscopes can magnify effectively to about 1,000 times the actual size of the specimen; at greater magnifications, additional details cannot be seen clearly. *Resolution* is a measure of the clarity of the image; it is the minimum distance two points can be separated and still be distinguished as separate points. For example, what appears to the unaided eye as one star in the sky may be resolved as twin stars with a telescope, which has a higher resolving ability than the eye. Similarly, using standard techniques, the light microscope cannot resolve detail finer than about 0.2 micrometer (μm), or 200 nanometers (nm), regardless of the magnification (**Figure 7.2**). The third parameter, *contrast*, is the difference in brightness between the light and dark areas of an image. Methods for enhancing contrast include staining or labeling cell components to stand out visually. **Figure 7.3** shows some different types of microscopy; study this figure as you read this section.

Until recently, the resolution barrier prevented cell biologists from using standard light microscopy when studying **organelles**, the membrane-enclosed structures within eukaryotic cells. To see these structures in any detail required the development of a new instrument. In the 1950s, the electron microscope was introduced to biology. Rather than focusing light, the **electron microscope (EM)** focuses a

▼ **Figure 7.2 The size range of cells.** Most cells are between 1 and 100 μm in diameter (yellow region of chart) and their components are even smaller (see Figure 7.32), as are viruses. Notice that the scale along the left side is logarithmic, to accommodate the range of sizes shown. Starting at the top of the scale with 10 m and going down, each reference measurement marks a tenfold decrease in diameter or length. For a complete table of the metric system, see Appendix C.



1 centimeter (cm) = 10^{-2} meter (m) = 0.4 inch

1 millimeter (mm) = 10^{-3} m

1 micrometer (μm) = 10^{-3} mm = 10^{-6} m

1 nanometer (nm) = 10^{-3} μm = 10^{-9} m



Animation: Metric System Review

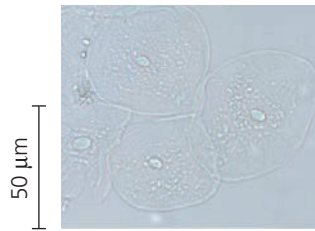
beam of electrons through the specimen or onto its surface (see Appendix D). Resolution is inversely related to the wavelength of the light (or electrons) a microscope uses for imaging, and electron beams have much shorter wavelengths than visible light. Modern electron microscopes can theoretically achieve a resolution of about 0.002 nm, though in practice they usually cannot resolve structures smaller than about 2 nm across. Still, this is a 100-fold improvement over the standard light microscope.

The **scanning electron microscope (SEM)** is especially useful for detailed study of the topography

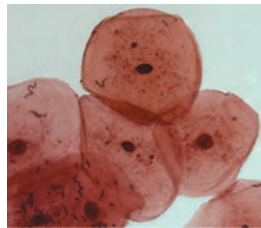
▼ Figure 7.3 Exploring Microscopy

Light Microscopy (LM)

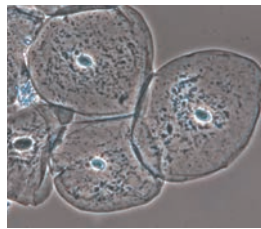
Brightfield (unstained specimen). Light passes directly through the specimen. Unless the cell is naturally pigmented or artificially stained, the image has little contrast. (The first four light micrographs show human cheek epithelial cells; the scale bar pertains to all four micrographs.)



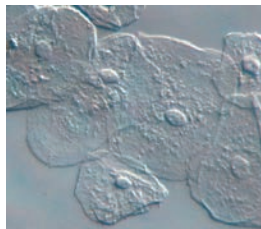
Brightfield (stained specimen). Staining with various dyes enhances contrast. Most staining procedures require that cells be fixed (preserved), thereby killing them.



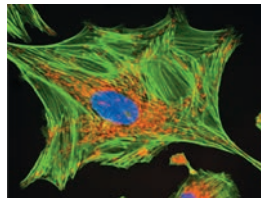
Phase-contrast. Variations in density within the specimen are amplified to enhance contrast in unstained cells; this is especially useful for examining living, unpigmented cells.



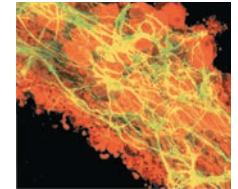
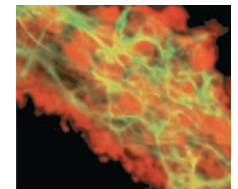
Differential interference contrast (Nomarski). As in phase-contrast microscopy, optical modifications are used to exaggerate differences in density; the image appears almost 3-D.



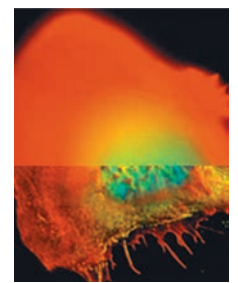
Fluorescence. The locations of specific molecules in the cell can be revealed by labeling the molecules with fluorescent dyes or antibodies; some cells have molecules that fluoresce on their own. Fluorescent substances absorb ultraviolet radiation and emit visible light. In this fluorescently labeled uterine cell, nuclear material is blue, organelles called mitochondria are orange, and the cell's "skeleton" is green.



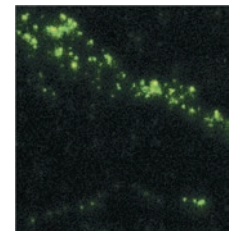
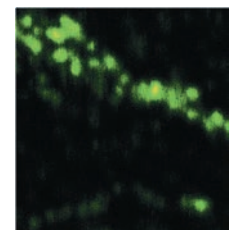
Confocal. The top image is a standard fluorescence micrograph of fluorescently labeled nervous tissue (nerve cells are green, support cells are orange, and regions of overlap are yellow); below it is a confocal image of the same tissue. Using a laser, this "optical sectioning" technique eliminates out-of-focus light from a thick sample, creating a single plane of fluorescence in the image. By capturing sharp images at many different planes, a 3-D reconstruction can be created. The standard image is blurry because out-of-focus light is not excluded.



Deconvolution. The top of this split image is a compilation of standard fluorescence micrographs through the depth of a white blood cell. Below is an image of the same cell reconstructed from many blurry images at different planes, each of which was processed using deconvolution software. This process digitally removes out-of-focus light and reassigns it to its source, creating a much sharper 3-D image.

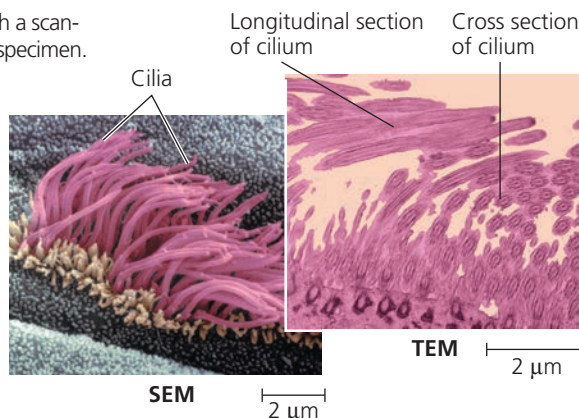


Super-resolution. On the top is a confocal image of part of a nerve cell, using a fluorescent label that binds to a molecule clustered in small sacs in the cell (vesicles) that are 40 nm in diameter. The greenish-yellow spots are blurry because 40 nm is below the 200-nm limit of resolution for standard light microscopy. Below is an image of the same part of the cell, seen using a new super-resolution technique. Sophisticated equipment is used to light up individual fluorescent molecules and record their position. Combining information from many molecules in different places "breaks" the limit of resolution, resulting in the sharp greenish-yellow dots seen here. (Each dot is a 40-nm vesicle.)



Electron Microscopy (EM)

Scanning electron microscopy (SEM). Micrographs taken with a scanning electron microscope show a 3-D image of the surface of a specimen. This SEM shows the surface of a cell from a trachea (windpipe) covered with cell projections called cilia. Electron micrographs are black and white but are often artificially colored to highlight particular structures, as has been done with both electron micrographs shown here.



Transmission electron microscopy (TEM).

A transmission electron microscope profiles a thin section of a specimen. This TEM shows a section through a tracheal cell, revealing its internal structure. In preparing the specimen, some cilia were cut along their lengths, creating longitudinal sections, while other cilia were cut straight across, creating cross sections.

Abbreviations used in figure legends in this text:
LM = Light Micrograph
SEM = Scanning Electron Micrograph
TEM = Transmission Electron Micrograph

VISUAL SKILLS ► When the tissue was sliced for the TEM, what was the orientation of the cilia in the upper left? On the right? Explain how the orientation determined the type of section we see.

of a specimen (see Figure 7.3). The electron beam scans the surface of the sample, usually coated with a thin film of gold. The beam excites electrons on the surface, and these secondary electrons are detected by a device that translates the pattern of electrons into an electronic signal sent to a video screen. The result is an image of the specimen's surface that appears three-dimensional.

The **transmission electron microscope (TEM)** is used to study the internal structure of cells (see Figure 7.3). The TEM aims an electron beam through a very thin section of the specimen, much as a light microscope aims light through a sample on a slide. For the TEM, the specimen has been stained with atoms of heavy metals, which attach to certain cellular structures, thus enhancing the electron density of some parts of the cell more than others. The electrons passing through the specimen are scattered more in the denser regions, so fewer are transmitted. The image displays the pattern of transmitted electrons. Instead of using glass lenses, both the SEM and TEM use electromagnets as lenses to bend the paths of the electrons, ultimately focusing the image onto a monitor for viewing.

Electron microscopes have revealed many subcellular structures that were impossible to resolve with the light microscope. But the light microscope offers advantages, especially in studying living cells. A disadvantage of electron microscopy is that the methods used to prepare the specimen kill the cells. Specimen preparation for any type of microscopy can introduce artifacts, structural features seen in micrographs that do not exist in the living cell.

In the past several decades, light microscopy has been revitalized by major technical advances (see Figure 7.3). Labeling individual cellular molecules or structures with fluorescent markers has made it possible to see such structures with increasing detail. In addition, both confocal and deconvolution microscopy have produced sharper images of three-dimensional tissues and cells. Finally, a group of new techniques and labeling molecules developed in recent years has allowed researchers to “break” the resolution barrier and distinguish subcellular structures even as small as 10–20 nm across. As this *super-resolution microscopy* becomes more widespread, the images we see of living cells are proving as exciting for us as van Leeuwenhoek’s were for Robert Hooke 350 years ago.

Microscopes are the most important tools of *cytology*, the study of cell structure. Understanding the function of each structure, however, required the integration of cytology and *biochemistry*, the study of the chemical processes (metabolism) of cells.

Cell Fractionation

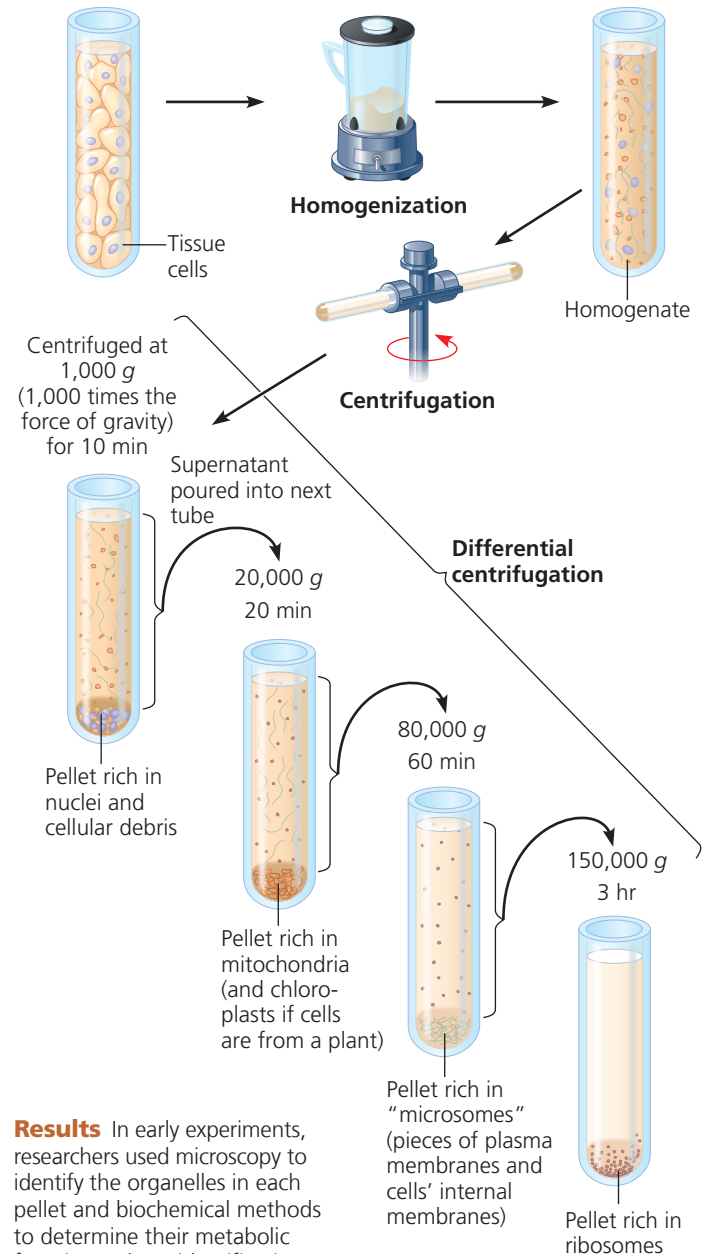
A useful technique for studying cell structure and function is **cell fractionation** (Figure 7.4), which takes cells apart and separates major organelles and other subcellular

▼ Figure 7.4

Research Method Cell Fractionation

Application Cell fractionation is used to separate (fractionate) cell components based on size and density.

Technique Cells are homogenized in a blender to break them up. The resulting mixture (homogenate) is centrifuged. The supernatant (the liquid above the pellet) is poured into another tube and centrifuged at a higher speed for a longer period. This process is repeated several times. This “differential centrifugation” results in a series of pellets, each containing different cell components.



Results In early experiments, researchers used microscopy to identify the organelles in each pellet and biochemical methods to determine their metabolic functions. These identifications established a baseline for this method, enabling today's researchers to know which cell fraction they should collect in order to isolate and study particular organelles.

MAKE CONNECTIONS ► If you wanted to study the process of translation of proteins from mRNA, which part of which fraction would you use? (See Figure 5.22.)

structures from one another. The piece of equipment that is used for this task is the centrifuge, which spins test tubes holding mixtures of disrupted cells at a series of increasing speeds. At each speed, the resulting force causes a subset of the cell components to settle to the bottom of the tube, forming a pellet. At lower speeds, the pellet consists of larger components, and higher speeds result in a pellet with smaller components.

Cell fractionation enables researchers to prepare specific cell components in bulk and identify their functions, a task not usually possible with intact cells. For example, on one of the cell fractions, biochemical tests showed the presence of enzymes involved in cellular respiration, while electron microscopy revealed large numbers of the organelles called mitochondria. Together, these data helped biologists determine that mitochondria are the sites of cellular respiration. Biochemistry and cytology thus complement each other in correlating cell function with structure.

CONCEPT CHECK 7.1

1. How do stains used for light microscopy compare with those used for electron microscopy?
2. **WHAT IF? >** Which type of microscope would you use to study (a) the internal structure of a plant cell and (b) fluorescently labeled nervous tissue?

For suggested answers, see Appendix A.

CONCEPT 7.2

Eukaryotic cells have internal membranes that compartmentalize their functions

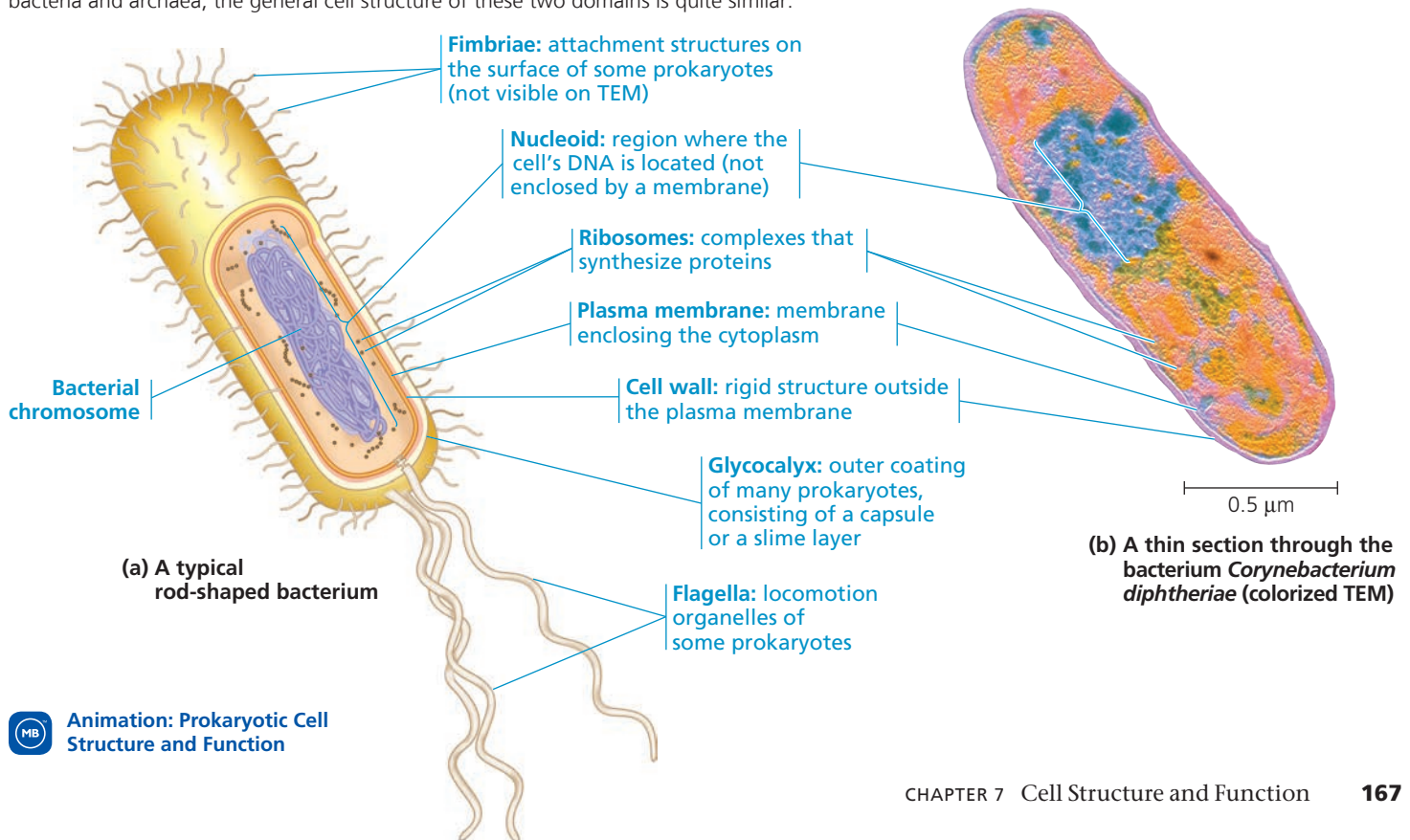
Cells—the basic structural and functional units of every organism—are of two distinct types: prokaryotic and eukaryotic. Organisms of the domains Bacteria and Archaea consist of prokaryotic cells. Protists, fungi, animals, and plants all consist of eukaryotic cells. (“Protist” is an informal term referring to a diverse group of mostly unicellular eukaryotes.)

Comparing Prokaryotic and Eukaryotic Cells

All cells share certain basic features: They are all bounded by a selective barrier, called the *plasma membrane* (also referred to as the cell membrane). Inside all cells is a semifluid, jellylike substance called **cytosol**, in which subcellular components are suspended. All cells contain *chromosomes*, which carry genes in the form of DNA. And all cells have *ribosomes*, tiny complexes that make proteins according to instructions from the genes.

A major difference between prokaryotic and eukaryotic cells is the location of their DNA. In a **eukaryotic cell**, most of the DNA is in an organelle called the *nucleus*, which is bounded by a double membrane (see Figure 7.8). In a **prokaryotic cell**, the DNA is concentrated in a region that is not membrane-enclosed, called the **nucleoid** (Figure 7.5).

▼ **Figure 7.5 A prokaryotic cell.** Lacking a true nucleus and the other membrane-enclosed organelles of the eukaryotic cell, the prokaryotic cell appears much simpler in internal structure. Prokaryotes include bacteria and archaea; the general cell structure of these two domains is quite similar.



Eukaryotic means “true nucleus” (from the Greek *eu*, true, and *karyon*, kernel, referring to the nucleus), and *prokaryotic* means “before nucleus” (from the Greek *pro*, before), reflecting the earlier evolution of prokaryotic cells.

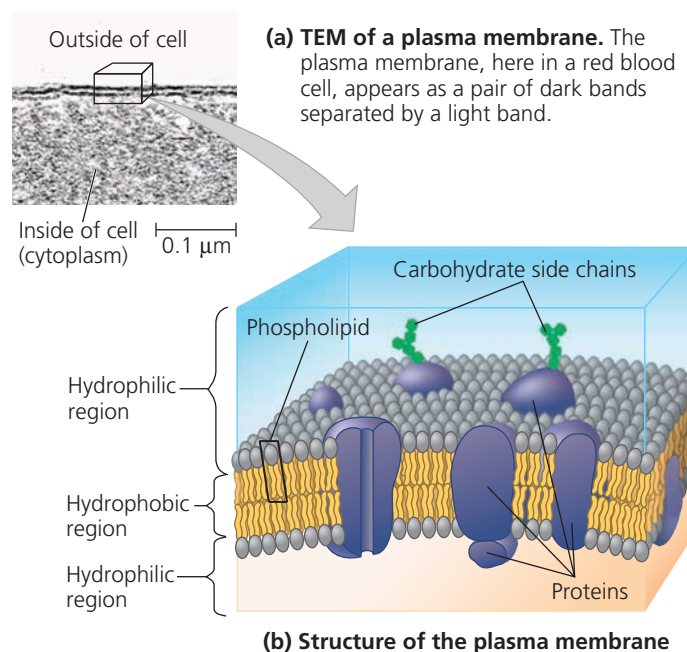
The interior of either type of cell is called the **cytoplasm**; in eukaryotic cells, this term refers only to the region between the nucleus and the plasma membrane. Within the cytoplasm of a eukaryotic cell, suspended in cytosol, are a variety of organelles of specialized form and function. These membrane-bounded structures are absent in almost all prokaryotic cells, another distinction between prokaryotic and eukaryotic cells. In spite of the absence of organelles, though, the prokaryotic cytoplasm is not a formless soup. For example, some prokaryotes contain regions surrounded by proteins (not membranes), within which specific reactions take place.

Eukaryotic cells are generally much larger than prokaryotic cells (see Figure 7.2). Size is a general feature of cell structure that relates to function. The logistics of carrying out cellular metabolism sets limits on cell size. At the lower limit, the smallest cells known are bacteria called mycoplasmas, which have diameters between 0.1 and 1.0 μm . These are perhaps the smallest packages with enough DNA to program metabolism and enough enzymes and other cellular equipment to carry out the activities necessary for a cell to sustain itself and reproduce. Typical bacteria are 1–5 μm in diameter, about ten times the size of mycoplasmas. Eukaryotic cells are typically 10–100 μm in diameter.

Metabolic requirements also impose theoretical upper limits on the size that is practical for a single cell. At the boundary of every cell, the **plasma membrane** functions as a selective barrier that allows passage of enough oxygen, nutrients, and wastes to service the entire cell (Figure 7.6). For each square micrometer of membrane, only a limited amount of a particular substance can cross per second, so the ratio of surface area to volume is critical. As a cell (or any other object) increases in size, its surface area grows proportionately less than its volume. (Area is proportional to a linear dimension squared, whereas volume is proportional to the linear dimension cubed.) Thus, a smaller object has a greater ratio of surface area to volume (Figure 7.7). The **Scientific Skills Exercise** gives you a chance to calculate the volumes and surface areas of two actual cells—a mature yeast cell and a cell budding from it. To see different ways organisms maximize the surface area of cells, see Make Connections Figure 33.9.

The need for a surface area large enough to accommodate the volume helps explain the microscopic size of most cells and the narrow, elongated shapes of others, such as nerve cells. Larger organisms do not generally have *larger* cells than smaller organisms—they simply have *more* cells (see Figure 7.7). A sufficiently high ratio of surface area to volume is especially important in cells that exchange a lot of material with their surroundings, such as intestinal cells. Such cells may have many long, thin projections from their surface called *microvilli*, which increase surface area without an appreciable increase in volume.

▼ **Figure 7.6 The plasma membrane.** The plasma membrane and the membranes of organelles consist of a double layer (bilayer) of phospholipids with various proteins attached to or embedded in it. The hydrophobic parts of phospholipids and membrane proteins are found in the interior of the membrane, while the hydrophilic parts are in contact with aqueous solutions on either side. Carbohydrate side chains may be attached to proteins or lipids on the outer surface of the plasma membrane.



VISUAL SKILLS ► What parts of the membrane diagram in (b) correspond to the dark bands, and which to the light band, in the TEM in (a)? (Review Figure 5.11.)

BioFlix® Animation: Membranes

▼ **Figure 7.7 Geometric relationships between surface area and volume.** In this diagram, cells are represented as boxes. Using arbitrary units of length, we can calculate the cell's surface area (in square units, or units²), volume (in cubic units, or units³), and ratio of surface area to volume. A high surface-to-volume ratio facilitates the exchange of materials between a cell and its environment.

	1	5	1
Total surface area [sum of the surface areas (height × width) of all box sides × number of boxes]	6	150	750
Total volume [height × width × length × number of boxes]	1	125	125
Surface-to-volume (S-to-V) ratio [surface area ÷ volume]	6	1.2	6

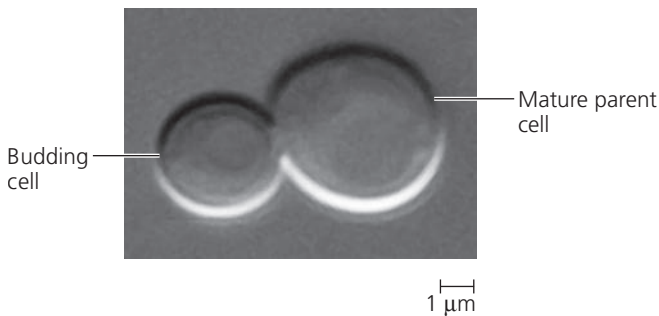
SCIENTIFIC SKILLS EXERCISE

Using a Scale Bar to Calculate Volume and Surface Area of a Cell

How Much New Cytoplasm and Plasma Membrane Are Made by a Growing Yeast Cell? The unicellular yeast *Saccharomyces cerevisiae* divides by budding off a small new cell that then grows to full size (see the yeast cells at the bottom of Figure 7.8). During its growth, the new cell synthesizes new cytoplasm, which increases its volume, and new plasma membrane, which increases its surface area. In this exercise, you will use a scale bar to determine the sizes of a mature parent yeast cell and a cell budding from it. You will then calculate the volume and surface area of each cell. You will use your calculations to determine how much cytoplasm and plasma membrane the new cell needs to synthesize to grow to full size.

How the Experiment Was Done Yeast cells were grown under conditions that promoted division by budding. The cells were then viewed with a differential interference contrast light microscope and photographed.

Data from the Experiment This light micrograph shows a budding yeast cell about to be released from the mature parent cell:



Micrograph from Kelly Tatchell, using yeast cells grown for experiments described in L. Kozubowski et al., Role of the septin ring in the asymmetric localization of proteins at the mother-bud neck in *Saccharomyces cerevisiae*, *Molecular Biology of the Cell* 16:3455–3466 (2005).

The evolutionary relationships between prokaryotic and eukaryotic cells will be discussed later in this chapter, and prokaryotic cells will be described in detail elsewhere (see Chapter 27). Most of the discussion of cell structure that follows in this chapter applies to eukaryotic cells.

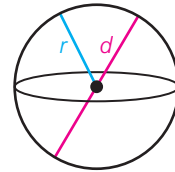
A Panoramic View of the Eukaryotic Cell

In addition to the plasma membrane at its outer surface, a eukaryotic cell has extensive, elaborately arranged internal membranes that divide the cell into compartments—the organelles mentioned earlier. The cell's compartments provide different local environments that support specific metabolic functions, so incompatible processes can occur simultaneously in a single cell. The plasma membrane and organelle membranes also participate directly in the cell's metabolism because many enzymes are built right into the membranes.

INTERPRET THE DATA

- Examine the micrograph of the yeast cells. The scale bar under the photo is labeled 1 μm . The scale bar works in the same way as a scale on a map, where, for example, 1 inch equals 1 mile. In this case the bar represents one thousandth of a millimeter. Using the scale bar as a basic unit, determine the diameter of the mature parent cell and the new cell. Start by measuring the scale bar and the diameter of each cell. The units you use are irrelevant, but working in millimeters is convenient. Divide each diameter by the length of the scale bar and then multiply by the scale bar's length value to give you the diameter in micrometers.
- The shape of a yeast cell can be approximated by a sphere. (a) Calculate the volume of each cell using the formula for the volume of a sphere:

$$V = \frac{4}{3}\pi r^3$$



Note that π (the Greek letter pi) is a constant with an approximate value of 3.14, d stands for diameter, and r stands for radius, which is half the diameter. (b) What volume of new cytoplasm will the new cell have to synthesize as it matures? To determine this, calculate the difference between the volume of the full-sized cell and the volume of the new cell.

- As the new cell grows, its plasma membrane needs to expand to contain the increased volume of the cell. (a) Calculate the surface area of each cell using the formula for the surface area of a sphere: $A = 4\pi r^2$. (b) How much area of new plasma membrane will the new cell have to synthesize as it matures?
- When the new cell matures, it will be approximately how many times greater in volume and how many times greater in surface area than its current size?

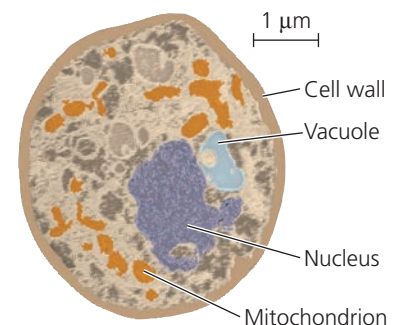
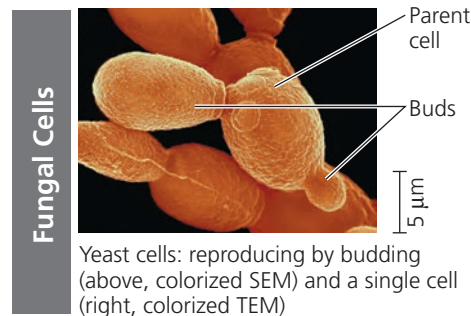
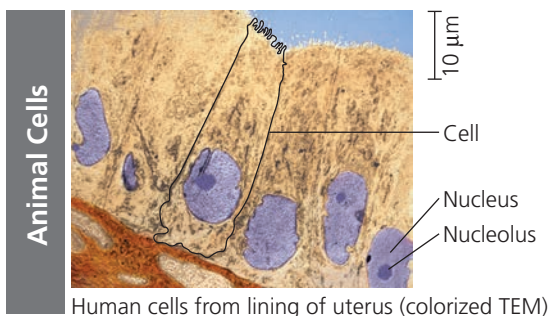
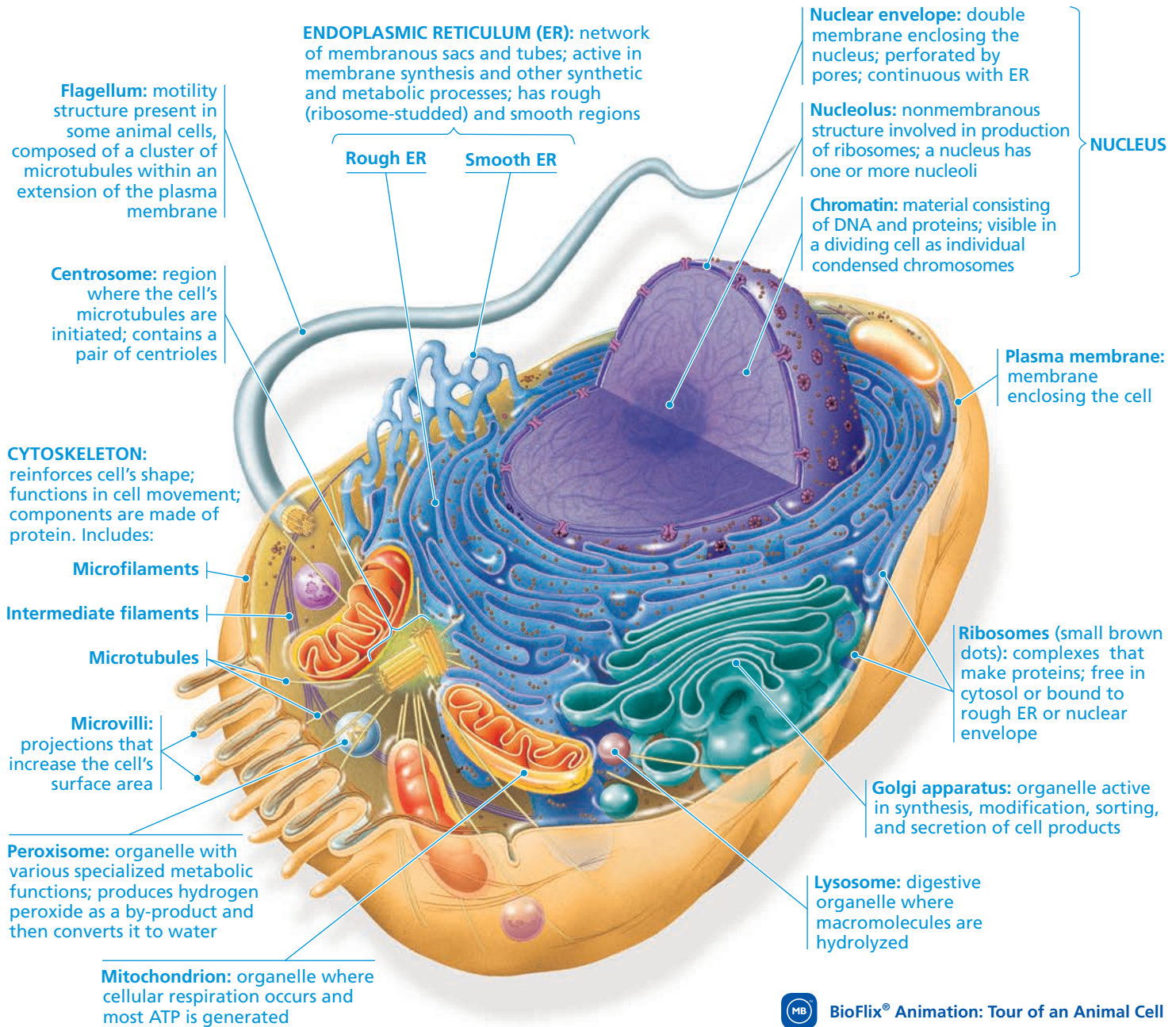


Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

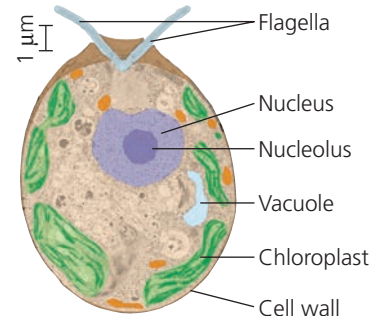
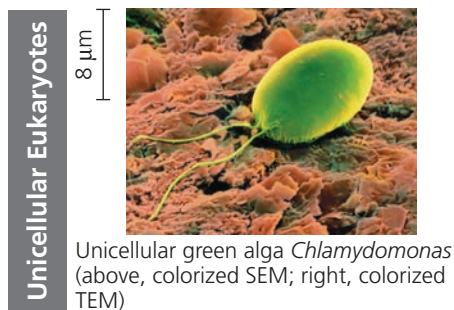
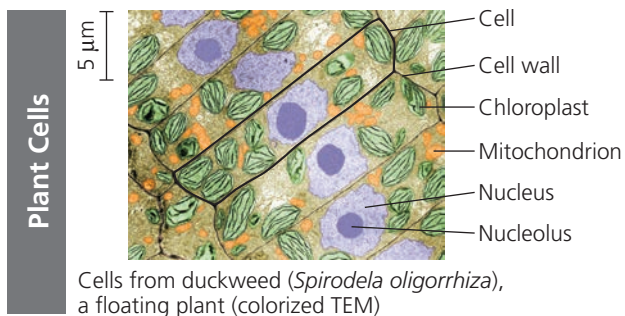
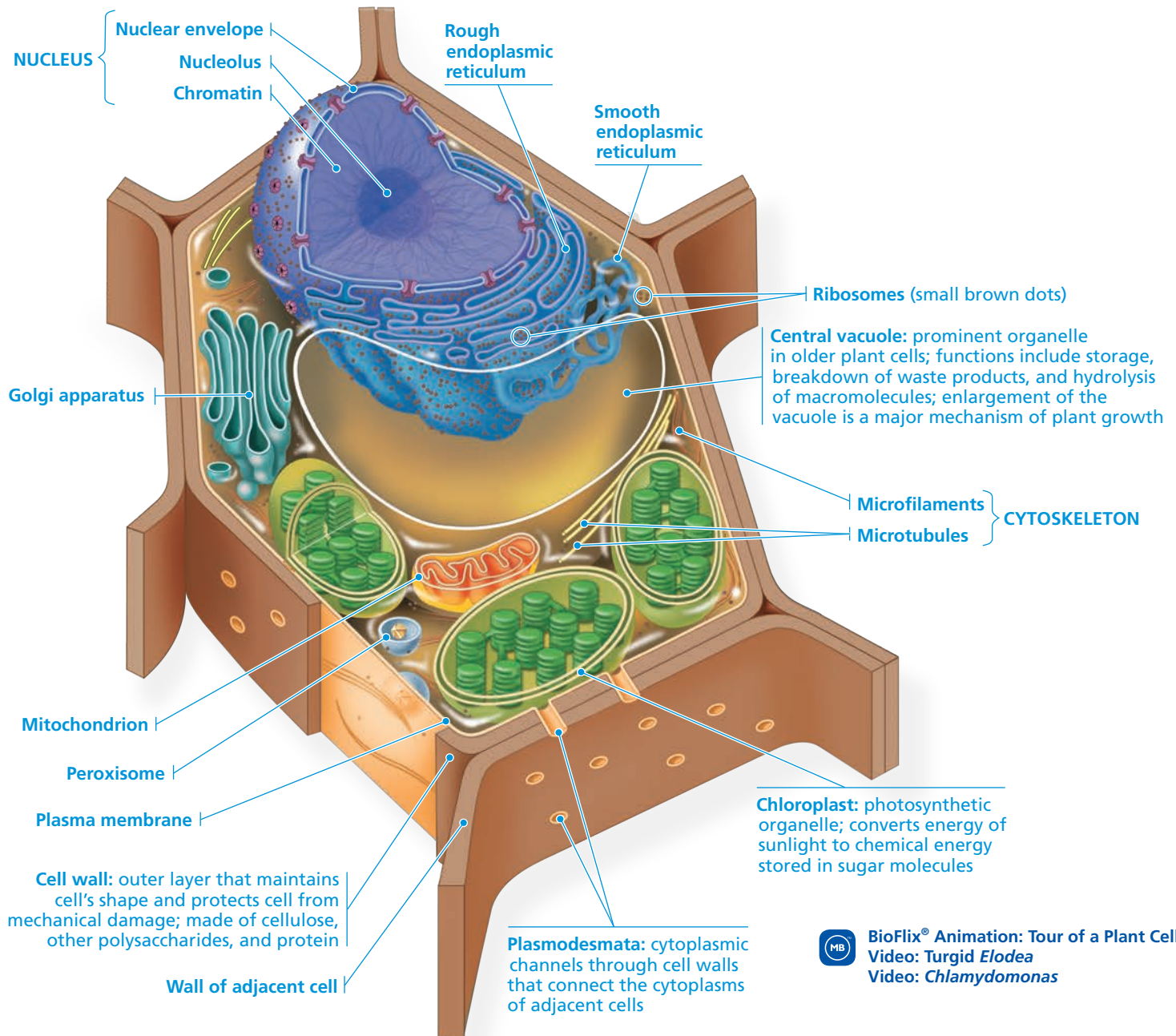
The basic fabric of most biological membranes is a double layer of phospholipids and other lipids. Embedded in this lipid bilayer or attached to its surfaces are diverse proteins (see Figure 7.6). However, each type of membrane has a unique composition of lipids and proteins suited to that membrane's specific functions. For example, enzymes embedded in the membranes of the organelles called mitochondria function in cellular respiration. Because membranes are so fundamental to the organization of the cell, Chapter 8 will discuss them in detail.

Before continuing with this chapter, examine the eukaryotic cells in **Figure 7.8**. The generalized diagrams of an animal cell and a plant cell introduce the various organelles and show the key differences between animal and plant cells. The micrographs at the bottom of the figure give you a glimpse of cells from different types of eukaryotic organisms.

Animal Cell (cutaway view of generalized cell)



Plant Cell (cutaway view of generalized cell)



CONCEPT CHECK 7.2

1. Briefly describe the structure and function of the nucleus, the mitochondrion, the chloroplast, and the endoplasmic reticulum.
2. **DRAW IT** ➤ Draw a simplified elongated cell that measures $125 \times 1 \times 1$ arbitrary units. A nerve cell would be roughly this shape. Predict how its surface-to-volume ratio would compare with those in Figure 7.7. Then calculate the ratio and check your prediction.

For suggested answers, see Appendix A.

CONCEPT 7.3

The eukaryotic cell's genetic instructions are housed in the nucleus and carried out by the ribosomes

On the first stop of our detailed tour of the eukaryotic cell, let's look at two cellular components involved in the genetic control of the cell: the nucleus, which houses most of the cell's DNA, and the ribosomes, which use information from the DNA to make proteins.

The Nucleus: Information Central

The **nucleus** contains most of the genes in the eukaryotic cell. (Some genes are located in mitochondria and chloroplasts.) It is usually the most conspicuous organelle (see the purple structure in the fluorescence micrograph), averaging about $5 \mu\text{m}$ in diameter. The **nuclear envelope** encloses the nucleus (Figure 7.9), separating its contents from the cytoplasm.

The nuclear envelope is a *double* membrane. The two membranes, each a lipid bilayer with associated proteins, are separated by a space of 20–40 nm. The envelope is perforated by pore structures that are about 100 nm in diameter. At the lip of each pore, the inner and outer membranes of the nuclear envelope are continuous. An intricate protein structure called a *pore complex* lines each pore and plays an important role in the cell by regulating the entry and exit of proteins and RNAs, as well as large complexes of macromolecules. Except at the pores, the nuclear side of the envelope is lined by the **nuclear lamina**, a netlike array of protein filaments (in animal cells, called *intermediate filaments*) that maintains the shape of the nucleus by mechanically supporting the nuclear envelope. There is also much evidence for a *nuclear matrix*, a framework of protein fibers extending throughout the nuclear interior. The nuclear lamina and matrix may help organize the genetic material so it functions efficiently.

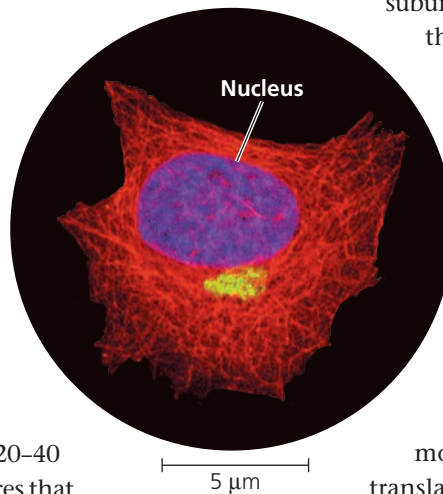
Within the nucleus, the DNA is organized into discrete units called **chromosomes**, structures that carry the genetic information. Each chromosome contains one long DNA molecule

associated with many proteins. Some of the proteins help coil the DNA molecule of each chromosome, reducing its length and allowing it to fit into the nucleus. The complex of DNA and proteins making up chromosomes is called **chromatin**. When a cell is not dividing, stained chromatin appears as a diffuse mass in micrographs, and the chromosomes cannot be distinguished from one another, even though discrete chromosomes are present. As a cell prepares to divide, however, the chromosomes coil (condense) further, becoming thick enough to be distinguished under a microscope as separate structures. Each eukaryotic species has a characteristic number of chromosomes. For example, a typical human cell has 46 chromosomes in its nucleus; the exceptions are the sex cells (eggs and sperm), which have only 23 chromosomes in humans. A fruit fly cell has 8 chromosomes in most cells and 4 in the sex cells.

A prominent structure within the nondividing nucleus is the **nucleolus** (plural, *nucleoli*), which appears through the electron microscope as a mass of densely stained granules and fibers adjoining part of the chromatin. Here a type of RNA called *ribosomal RNA* (rRNA) is synthesized from instructions in the DNA. Also in the nucleolus, proteins imported from the

cytoplasm are assembled with rRNA into large and small subunits of ribosomes. These subunits then exit the nucleus through the nuclear pores to the cytoplasm, where a large and a small subunit can assemble into a ribosome. Sometimes there are two or more nucleoli; the number depends on the species and the stage in the cell's reproductive cycle.

As we saw in Figure 5.22, the nucleus directs protein synthesis by synthesizing messenger RNA (mRNA) according to instructions provided by the DNA. The mRNA is then transported to the cytoplasm via the nuclear pores. Once an mRNA molecule reaches the cytoplasm, ribosomes translate the mRNA's genetic message into the primary structure of a specific polypeptide. (This process of transcribing and translating genetic information is described in detail in Chapter 17.)

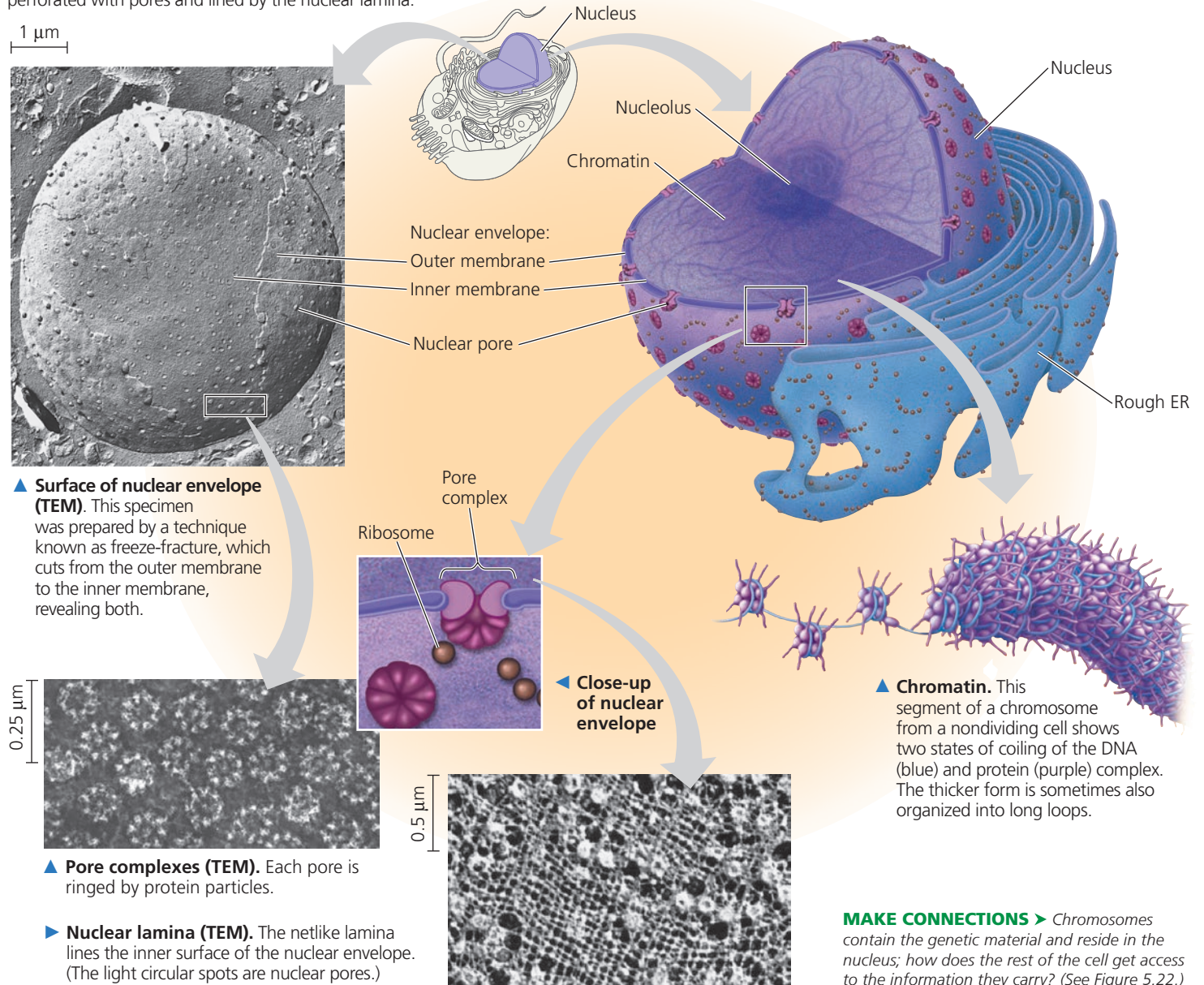


 **BioFlix® Animation: Nucleus and Ribosomes**

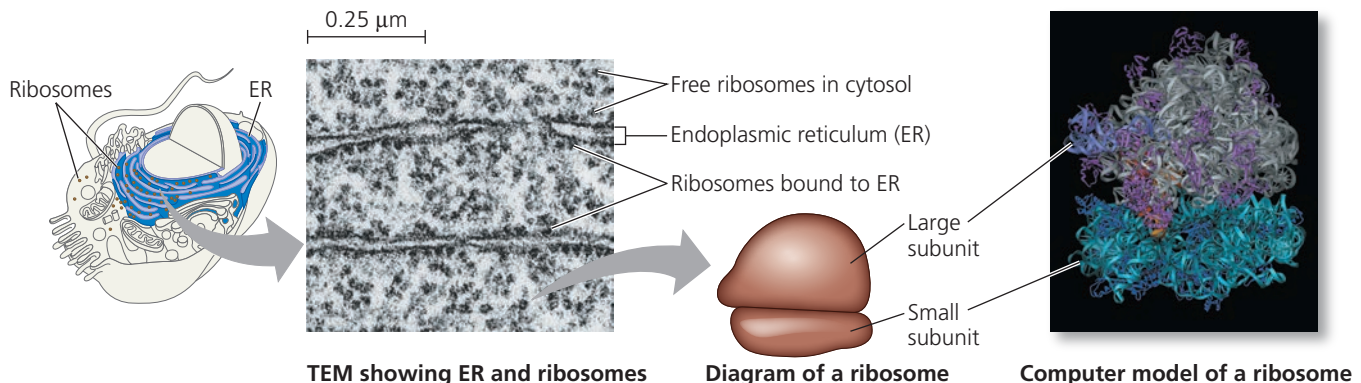
Ribosomes: Protein Factories

Ribosomes, which are complexes made of ribosomal RNAs and proteins, are the cellular components that carry out protein synthesis (Figure 7.10). (Note that ribosomes are not membrane bounded and thus are not considered organelles.) Cells that have high rates of protein synthesis have particularly large numbers of ribosomes as well as prominent nucleoli, which makes sense, given the role of nucleoli in ribosome assembly. For example, a human pancreas cell, which makes many digestive enzymes, has a few million ribosomes.

▼ **Figure 7.9 The nucleus and its envelope.** Within the nucleus are the chromosomes, which appear as a mass of chromatin (DNA and associated proteins) and one or more nucleoli (singular, *nucleolus*), which function in ribosome synthesis. The nuclear envelope, which consists of two membranes separated by a narrow space, is perforated with pores and lined by the nuclear lamina.



▼ **Figure 7.10 Ribosomes.** This electron micrograph of a pancreas cell shows both free and bound ribosomes. The simplified diagram and computer model show the two subunits of a ribosome.



DRAW IT ► After you have read the section on ribosomes, circle a ribosome in the micrograph that might be making a protein that will be secreted.

Interview with Venki Ramakrishnan: Studying ribosome structure

Ribosomes build proteins in two cytoplasmic locales. At any given time, *free ribosomes* are suspended in the cytosol, while *bound ribosomes* are attached to the outside of the endoplasmic reticulum or nuclear envelope (see Figure 7.10). Bound and free ribosomes are structurally identical, and ribosomes can play either role at different times. Most of the proteins made on free ribosomes function within the cytosol; examples are enzymes that catalyze the first steps of sugar breakdown. Bound ribosomes generally make proteins that are destined for insertion into membranes, for packaging within certain organelles such as lysosomes (see Figure 7.8), or for export from the cell (secretion). Cells that specialize in protein secretion—for instance, the cells of the pancreas that secrete digestive enzymes—frequently have a high proportion of bound ribosomes. (You will learn more about ribosome structure and function in Concept 17.4.)

CONCEPT CHECK 7.3

1. What role do ribosomes play in carrying out genetic instructions?
2. Describe the molecular composition of nucleoli and explain their function.
3. **WHAT IF? >** As a cell begins the process of dividing, its chromosomes become shorter, thicker, and individually visible in an LM (light micrograph). Explain what is happening at the molecular level.

For suggested answers, see Appendix A.

CONCEPT 7.4

The endomembrane system regulates protein traffic and performs metabolic functions

Many of the different membrane-bounded organelles of the eukaryotic cell are part of the **endomembrane system**, which includes the nuclear envelope, the endoplasmic reticulum, the Golgi apparatus, lysosomes, various kinds of vesicles and vacuoles, and the plasma membrane. This system carries out a variety of tasks in the cell, including synthesis of proteins, transport of proteins into membranes and organelles or out of the cell, metabolism and movement of lipids, and detoxification of poisons. The membranes of this system are related either through direct physical continuity or by the transfer of membrane segments as tiny **vesicles** (sacs made of membrane). Despite these relationships, the various membranes are not identical in structure and function. Moreover, the thickness, molecular composition, and types of chemical reactions carried out in a given membrane are not fixed, but may be modified several times during the membrane's life. Having already discussed the nuclear envelope, we will now focus on the endoplasmic reticulum and the other endomembranes to which the endoplasmic reticulum gives rise.

The Endoplasmic Reticulum: Biosynthetic Factory

The **endoplasmic reticulum (ER)** is such an extensive network of membranes that it accounts for more than half the total membrane in many eukaryotic cells. (The word *endoplasmic* means “within the cytoplasm,” and *reticulum* is Latin for “little net.”) The ER consists of a network of membranous tubules and sacs called cisternae (from the Latin *cisterna*, a reservoir for a liquid). The ER membrane separates the internal compartment of the ER, called the *ER lumen* (cavity) or cisternal space, from the cytosol. And because the ER membrane is continuous with the nuclear envelope, the space between the two membranes of the envelope is continuous with the lumen of the ER (**Figure 7.11**).

There are two distinct, though connected, regions of the ER that differ in structure and function: smooth ER and rough ER. **Smooth ER** is so named because its outer surface lacks ribosomes. **Rough ER** is studded with ribosomes on the outer surface of the membrane and thus appears rough through the electron microscope. As already mentioned, ribosomes are also attached to the cytoplasmic side of the nuclear envelope's outer membrane, which is continuous with rough ER.

Functions of Smooth ER

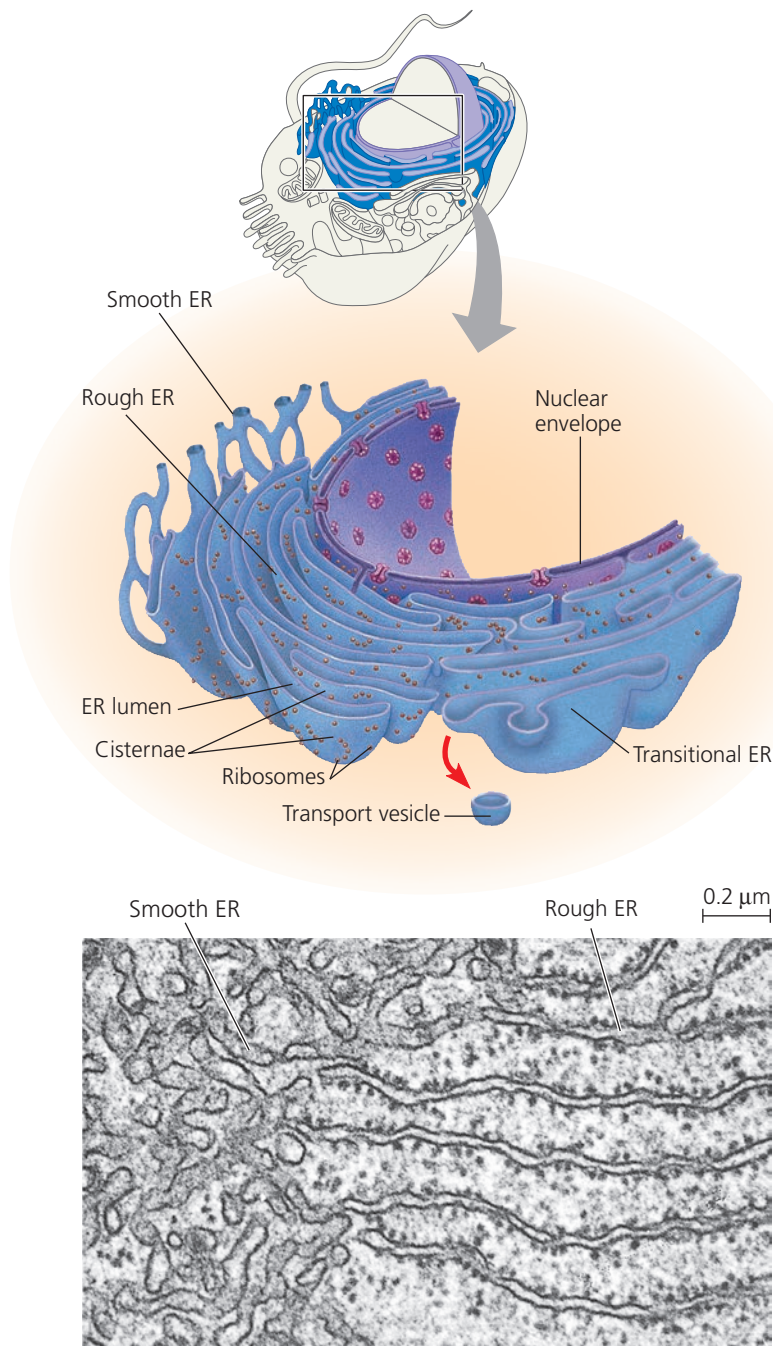
The smooth ER functions in diverse metabolic processes, which vary with cell type. These processes include synthesis of lipids, metabolism of carbohydrates, detoxification of drugs and poisons, and storage of calcium ions.

Enzymes of the smooth ER are important in the synthesis of lipids, including oils, steroids, and new membrane phospholipids. Among the steroids produced by the smooth ER in animal cells are the sex hormones of vertebrates and the various steroid hormones secreted by the adrenal glands. The cells that synthesize and secrete these hormones—in the testes and ovaries, for example—are rich in smooth ER, a structural feature that fits the function of these cells.

Other enzymes of the smooth ER help detoxify drugs and poisons, especially in liver cells. Detoxification usually involves adding hydroxyl groups to drug molecules, making them more soluble and easier to flush from the body. The sedative phenobarbital and other barbiturates are examples of drugs metabolized in this manner by smooth ER in liver cells. In fact, barbiturates, alcohol, and many other drugs induce the proliferation of smooth ER and its associated detoxification enzymes, thus increasing the rate of detoxification. This, in turn, increases tolerance to the drugs, meaning that higher doses are required to achieve a particular effect, such as sedation. Also, because some of the detoxification enzymes have relatively broad action,

the proliferation of smooth ER in response to one drug can increase the need for higher dosages of other drugs as well. Barbiturate abuse, for example, can decrease the effectiveness of certain antibiotics and other useful drugs.

▼ **Figure 7.11 Endoplasmic reticulum (ER).** A membranous system of interconnected tubules and flattened sacs called cisternae, the ER is also continuous with the nuclear envelope, as shown in the cutaway diagram at the top. The membrane of the ER encloses a continuous compartment called the ER lumen (or cisternal space). Rough ER, which is studded on its outer surface with ribosomes, can be distinguished from smooth ER in the electron micrograph (TEM). Transport vesicles bud off from a region of the rough ER called transitional ER and travel to the Golgi apparatus and other destinations.



The smooth ER also stores calcium ions. In muscle cells, for example, the smooth ER membrane pumps calcium ions from the cytosol into the ER lumen. When a muscle cell is stimulated by a nerve impulse, calcium ions rush back across the ER membrane into the cytosol and trigger contraction of the muscle cell. In other cell types, release of calcium ions from the smooth ER triggers different responses, such as secretion of vesicles carrying newly synthesized proteins.

Functions of Rough ER

Many cells secrete proteins that are produced by ribosomes attached to rough ER. For example, certain pancreatic cells synthesize the protein insulin in the ER and secrete this hormone into the bloodstream. As a polypeptide chain grows from a bound ribosome, the chain is threaded into the ER lumen through a pore formed by a protein complex in the ER membrane. The new polypeptide folds into its functional shape as it enters the ER lumen. Most secretory proteins are **glycoproteins**, proteins with carbohydrates covalently bonded to them. The carbohydrates are attached to the proteins in the ER lumen by enzymes built into the ER membrane.

After secretory proteins are formed, the ER membrane keeps them separate from proteins in the cytosol, which are produced by free ribosomes. Secretory proteins depart from the ER wrapped in the membranes of vesicles that bud like bubbles from a specialized region called transitional ER (see Figure 7.11). Vesicles in transit from one part of the cell to another are called **transport vesicles**; we will discuss their fate shortly.

In addition to making secretory proteins, rough ER is a membrane factory for the cell; it grows in place by adding membrane proteins and phospholipids to its own membrane. As polypeptides destined to be membrane proteins grow from the ribosomes, they are inserted into the ER membrane itself and anchored there by their hydrophobic portions. Like the smooth ER, the rough ER also makes membrane phospholipids; enzymes built into the ER membrane assemble phospholipids from precursors in the cytosol. The ER membrane expands, and portions of it are transferred in the form of transport vesicles to other components of the endomembrane system.

The Golgi Apparatus: Shipping and Receiving Center

After leaving the ER, many transport vesicles travel to the **Golgi apparatus**. We can think of the Golgi as a warehouse for receiving, sorting, shipping, and even some manufacturing. Here, products of the ER, such as proteins, are modified and stored and then sent to other destinations. Not surprisingly, the Golgi apparatus is especially extensive in cells specialized for secretion.

The Golgi apparatus consists of a group of associated, flattened membranous sacs—cisternae—looking like a stack of pita bread (**Figure 7.12**). A cell may have many, even hundreds, of these stacks. The membrane of each cisterna in a stack separates its internal space from the cytosol. Vesicles concentrated in the vicinity of the Golgi apparatus are engaged in the transfer of material between parts of the Golgi and other structures.

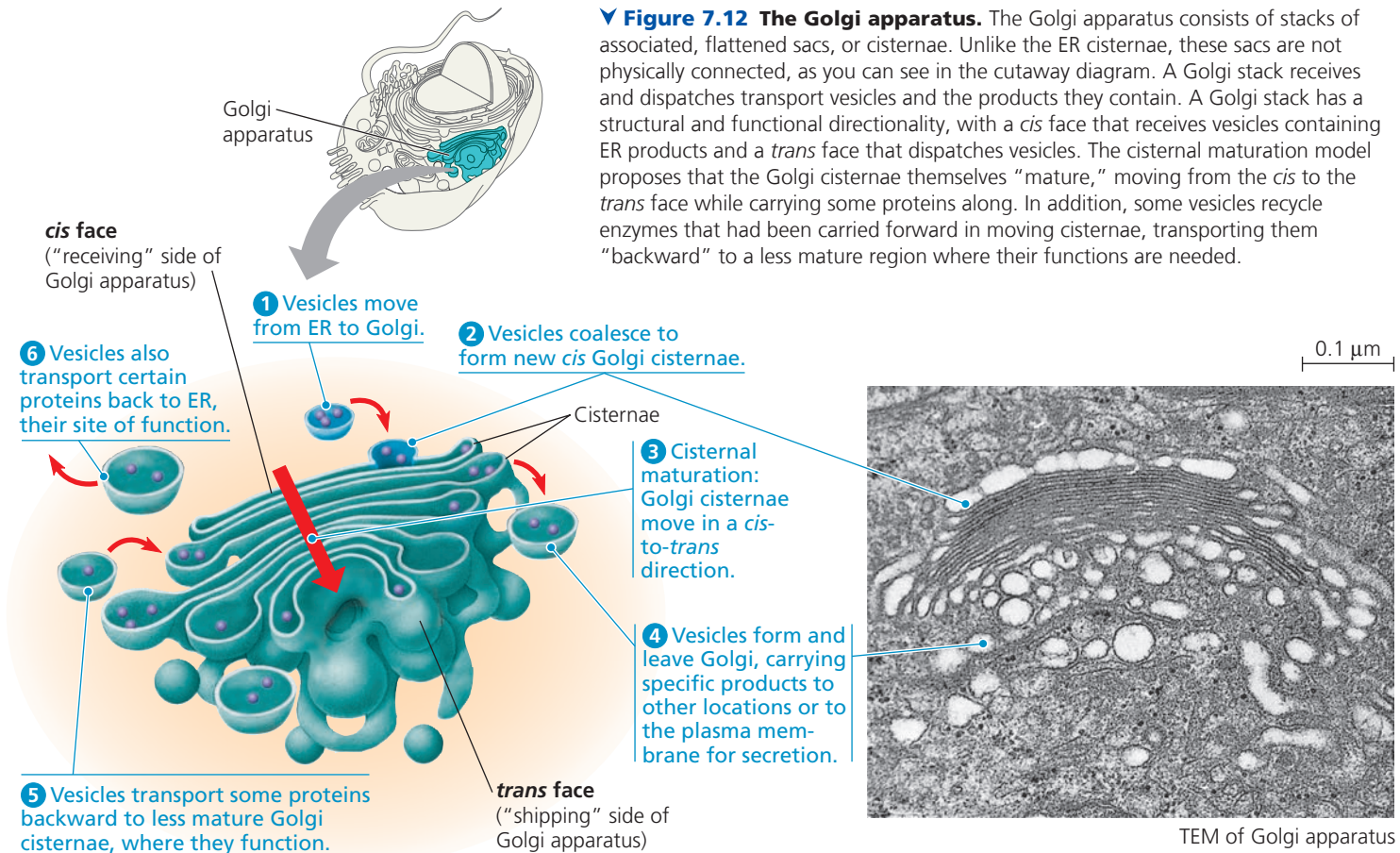
A Golgi stack has a distinct structural directionality, with the membranes of cisternae on opposite sides of the stack differing in thickness and molecular composition. The two sides of a Golgi stack are referred to as the *cis* face and the *trans* face; these act, respectively, as the receiving and shipping departments of the Golgi apparatus. The term *cis* means “on the same side,” and the *cis* face is usually located near the ER. Transport vesicles move material from the ER to the Golgi apparatus. A vesicle that buds from the ER can add its membrane and the contents of its lumen to the *cis* face by fusing with a Golgi membrane on that side. The *trans* face (“on the opposite side”) gives rise to vesicles that pinch off and travel to other sites.

Products of the endoplasmic reticulum are usually modified during their transit from the *cis* region to the *trans* region of the Golgi apparatus. For example, glycoproteins formed in the ER have their carbohydrates modified, first in the ER itself, and then as they pass through the Golgi. The Golgi

removes some sugar monomers and substitutes others, producing a large variety of carbohydrates. Membrane phospholipids may also be altered in the Golgi.

In addition to its finishing work, the Golgi apparatus also manufactures some macromolecules. Many polysaccharides secreted by cells are Golgi products. For example, pectins and certain other noncellulose polysaccharides are made in the Golgi of plant cells and then incorporated along with cellulose into their cell walls. Like secretory proteins, nonprotein Golgi products that will be secreted depart from the *trans* face of the Golgi inside transport vesicles that eventually fuse with the plasma membrane.

The Golgi manufactures and refines its products in stages, with different cisternae containing unique teams of enzymes. Until recently, biologists viewed the Golgi as a static structure, with products in various stages of processing transferred from one cisterna to the next by vesicles. While this may occur, research from several labs has given rise to a new model of the Golgi as a more dynamic structure. According to the *cisternal maturation model*, the cisternae of the Golgi actually progress forward from the *cis* to the *trans* face, carrying and modifying their cargo as they move. Figure 7.12 shows the details of this model. The reality probably lies somewhere between the two models; recent research suggests the central regions of the cisternae may remain in place, while the outer ends are more dynamic.



Before a Golgi stack dispatches its products by budding vesicles from the *trans* face, it sorts these products and targets them for various parts of the cell. Molecular identification tags, such as phosphate groups added to the Golgi products, aid in sorting by acting like zip codes on mailing labels. Finally, transport vesicles budded from the Golgi may have external molecules on their membranes that recognize “docking sites” on the surface of specific organelles or on the plasma membrane, thus targeting the vesicles appropriately.

Lysosomes: Digestive Compartments

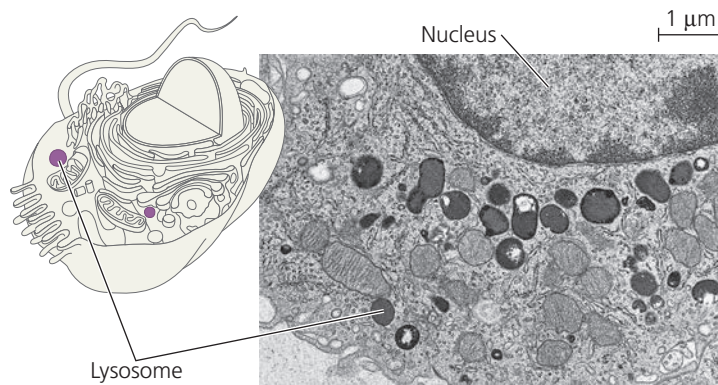
A **lysosome** is a membranous sac of hydrolytic enzymes that many eukaryotic cells use to digest (hydrolyze) macromolecules. Lysosomal enzymes work best in the acidic environment found in lysosomes. If a lysosome breaks open or leaks its contents, the released enzymes are not very active because the cytosol has a near-neutral pH. However, excessive leakage from a large number of lysosomes can destroy a cell by self-digestion.

Hydrolytic enzymes and lysosomal membrane are made by rough ER and then transferred to the Golgi apparatus for

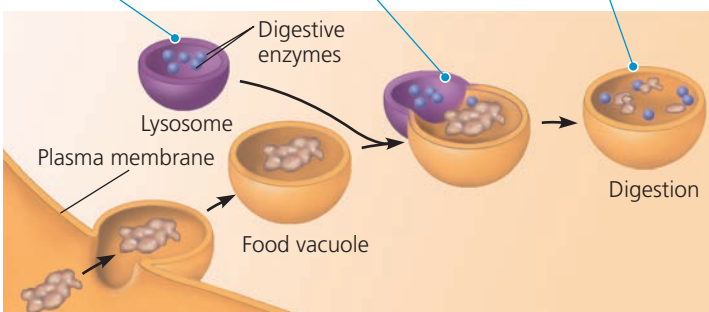
further processing. At least some lysosomes probably arise by budding from the *trans* face of the Golgi apparatus (see Figure 7.12). How are the proteins of the inner surface of the lysosomal membrane and the digestive enzymes themselves spared from destruction? Apparently, the three-dimensional shapes of these proteins protect vulnerable bonds from enzymatic attack.

Lysosomes carry out intracellular digestion in a variety of circumstances. Amoebas and many other unicellular eukaryotes eat by engulfing smaller organisms or food particles, a process called **phagocytosis** (from the Greek *phagein*, to eat, and *kytos*, vessel, referring here to the cell). The *food vacuole* formed in this way then fuses with a lysosome, whose enzymes digest the food (**Figure 7.13a**, bottom). Digestion products, including simple sugars, amino acids, and other monomers, pass into the cytosol and become nutrients for the cell. Some human cells also carry out phagocytosis. Among them are macrophages, a type of white blood cell that helps defend the body by engulfing and destroying bacteria and other invaders (see Figure 7.13a, top, and Figure 7.31).

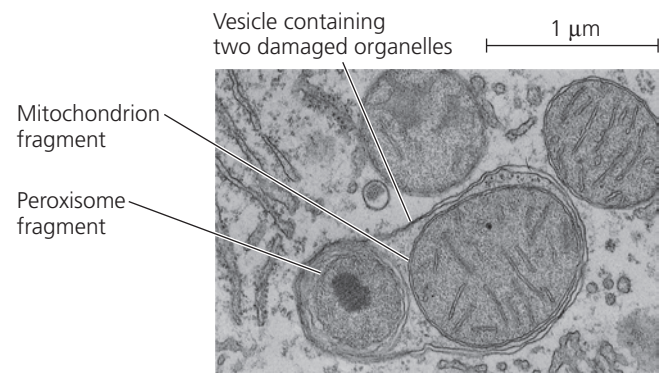
▼ **Figure 7.13 Lysosomes.**



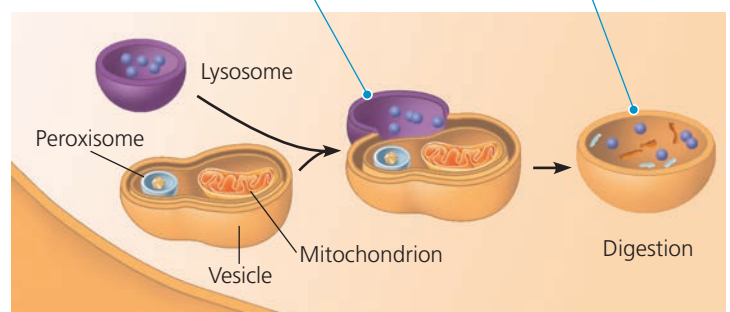
- 1 Lysosome contains active hydrolytic enzymes.
- 2 Lysosome fuses with food vacuole.
- 3 Hydrolytic enzymes digest food particles.



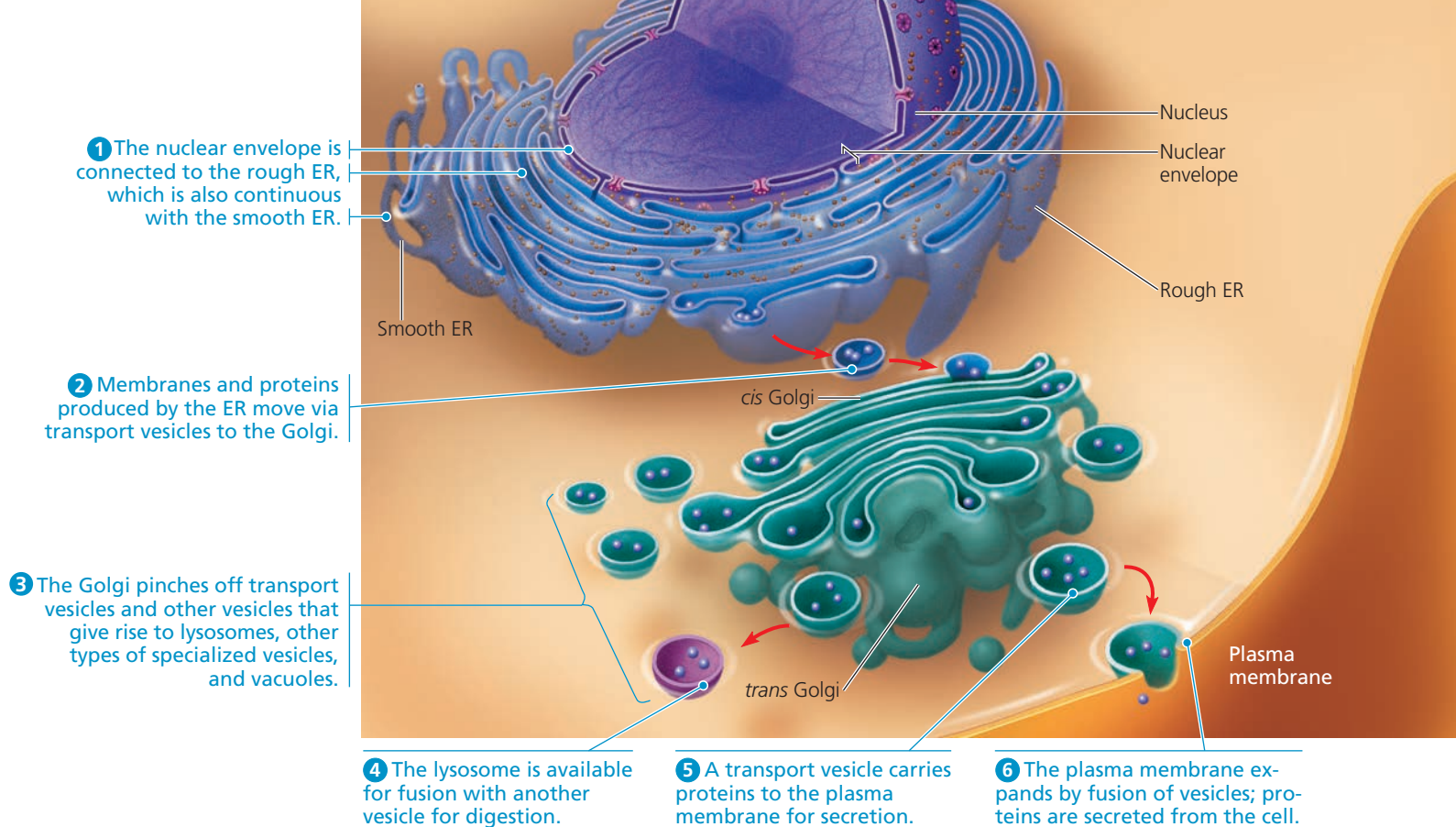
(a) Phagocytosis. In phagocytosis, lysosomes digest (hydrolyze) materials taken into the cell. *Top:* In this macrophage (a type of white blood cell) from a rat, the lysosomes are very dark because of a stain that reacts with one of the products of digestion inside the lysosome (TEM). Macrophages ingest bacteria and viruses and destroy them using lysosomes. *Bottom:* This diagram shows a lysosome fusing with a food vacuole during the process of phagocytosis by a unicellular eukaryote.



- 1 Lysosome fuses with vesicle containing damaged organelles.
- 2 Hydrolytic enzymes digest organelle components.



(b) Autophagy. In autophagy, lysosomes recycle intracellular materials. *Top:* In the cytoplasm of this rat liver cell is a vesicle containing two disabled organelles (TEM). The vesicle will fuse with a lysosome in the process of autophagy, which recycles intracellular materials. *Bottom:* This diagram shows fusion of such a vesicle with a lysosome. This type of vesicle has a double membrane of unknown origin. The outer membrane fuses with the lysosome, and the inner membrane is degraded along with the damaged organelles.



▲ Figure 7.15 Review: relationships among organelles of the endomembrane system. The red arrows show some of the migration pathways for membranes and the materials they enclose.

BioFlix® Animation:
Endomembrane System

CONCEPT 7.5

Mitochondria and chloroplasts change energy from one form to another

Organisms transform the energy they acquire from their surroundings. In eukaryotic cells, mitochondria and chloroplasts are the organelles that convert energy to forms that cells can use for work. **Mitochondria** (singular, *mitochondrion*) are the sites of cellular respiration, the metabolic process that uses oxygen to drive the generation of ATP by extracting energy from sugars, fats, and other fuels. **Chloroplasts**, found in plants and algae, are the sites of photosynthesis. This process in chloroplasts converts solar energy to chemical energy by absorbing sunlight and using it to drive the synthesis of organic compounds such as sugars from carbon dioxide and water.

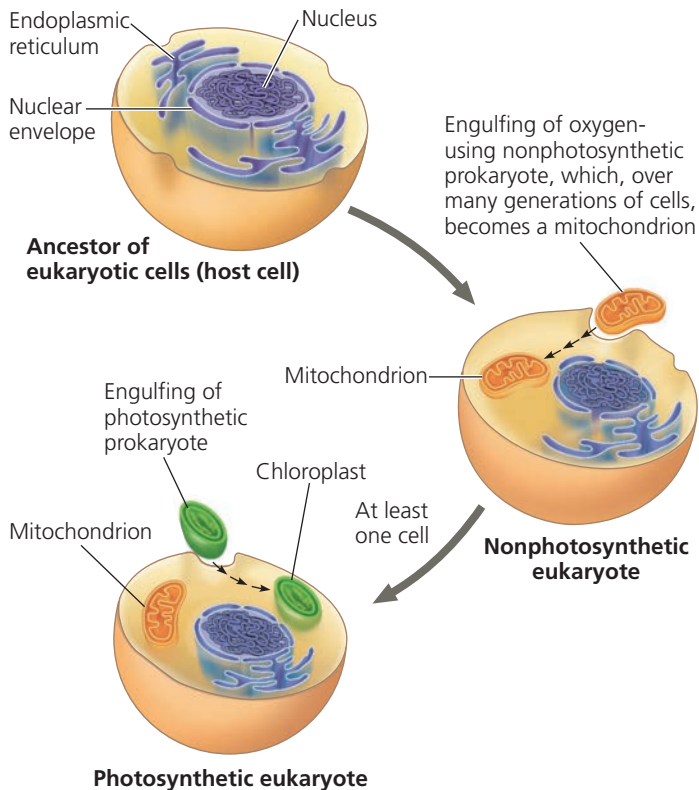
In addition to having related functions, mitochondria and chloroplasts share similar evolutionary origins, which we'll discuss briefly before describing their structures. In this section, we will also consider the peroxisome, an oxidative organelle. The evolutionary origin of the peroxisome, as well as its relation to other organelles, is still a matter of some debate.

The Evolutionary Origins of Mitochondria and Chloroplasts

EVOLUTION Mitochondria and chloroplasts display similarities with bacteria that led to the **endosymbiont theory**, illustrated in **Figure 7.16**. This theory states that an early ancestor of eukaryotic cells engulfed an oxygen-using nonphotosynthetic prokaryotic cell. Eventually, the engulfed cell formed a relationship with the host cell in which it was enclosed, becoming an *endosymbiont* (a cell living within another cell). Indeed, over the course of evolution, the host cell and its endosymbiont merged into a single organism, a eukaryotic cell with a mitochondrion. At least one of these cells may have then taken up a photosynthetic prokaryote, becoming the ancestor of eukaryotic cells that contain chloroplasts.

This is a widely accepted theory, which we will discuss in more detail in Concept 25.3. This theory is consistent with many structural features of mitochondria and chloroplasts. First, rather than being bounded by a single membrane like organelles of the endomembrane system, mitochondria and typical chloroplasts have two membranes surrounding them. (Chloroplasts also have an internal system of membranous sacs.) There is evidence that the ancestral engulfed

▼ **Figure 7.16 The endosymbiont theory of the origins of mitochondria and chloroplasts in eukaryotic cells.** According to this theory, the proposed ancestors of mitochondria were oxygen-using nonphotosynthetic prokaryotes, while the proposed ancestors of chloroplasts were photosynthetic prokaryotes. The large arrows represent change over evolutionary time; the small arrows inside the cells show the process of the endosymbiont becoming an organelle, also over long periods of time.



prokaryotes had two outer membranes, which became the double membranes of mitochondria and chloroplasts. Second, like prokaryotes, mitochondria and chloroplasts contain ribosomes, as well as circular DNA molecules—like bacterial chromosomes—associated with their inner membranes. The DNA in these organelles programs the synthesis of some organelle proteins on ribosomes that have been synthesized and assembled there as well. Third, also consistent with their probable evolutionary origins as cells, mitochondria and chloroplasts are autonomous (somewhat independent) organelles that grow and reproduce within the cell.

Next, we focus on the structures of mitochondria and chloroplasts, while providing an overview of their structures and functions. (In Chapters 10 and 11, we will examine their roles as energy transformers.)

Mitochondria: Chemical Energy Conversion

Mitochondria are found in nearly all eukaryotic cells, including those of plants, animals, fungi, and most unicellular eukaryotes. Some cells have a single large mitochondrion,

but more often a cell has hundreds or even thousands of mitochondria; the number correlates with the cell's level of metabolic activity. For example, cells that move or contract have proportionally more mitochondria per volume than less active cells.

Each of the two membranes enclosing the mitochondrion is a phospholipid bilayer with a unique collection of embedded proteins (**Figure 7.17**). The outer membrane is smooth, but the inner membrane is convoluted, with infoldings called **cristae**. The inner membrane divides the mitochondrion into two internal compartments. The first is the intermembrane space, the narrow region between the inner and outer membranes. The second compartment, the **mitochondrial matrix**, is enclosed by the inner membrane. The matrix contains many different enzymes as well as the mitochondrial DNA and ribosomes. Enzymes in the matrix catalyze some of the steps of cellular respiration. Other proteins that function in respiration, including the enzyme that makes ATP, are built into the inner membrane. As highly folded surfaces, the cristae give the inner mitochondrial membrane a large surface area, thus enhancing the productivity of cellular respiration. This is another example of structure fitting function.

Mitochondria are generally in the range of 1–10 μm long. Time-lapse films of living cells reveal mitochondria moving around, changing their shapes, and fusing or dividing in two, unlike the static structures seen in electron micrographs of dead cells. These studies helped cell biologists understand that mitochondria in a living cell form a branched tubular network, seen in a whole cell in **Figure 7.17b**, that is in a dynamic state of flux.

Chloroplasts: Capture of Light Energy

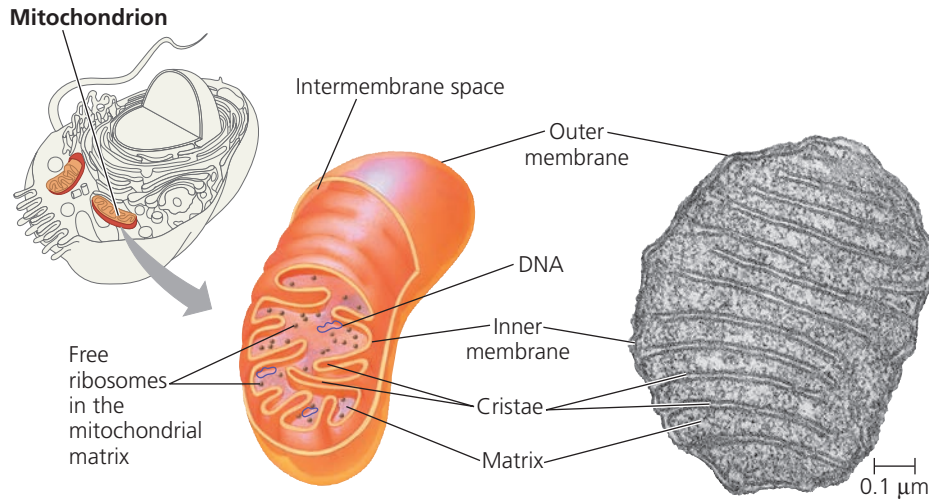
Chloroplasts contain the green pigment chlorophyll, along with enzymes and other molecules that function in the photosynthetic production of sugar. These lens-shaped organelles, about 3–6 μm in length, are found in leaves and other green organs of plants and in algae (**Figure 7.18**; see also **Figure 7.26c**).

The contents of a chloroplast are partitioned from the cytosol by an envelope consisting of two membranes separated by a very narrow intermembrane space. Inside the chloroplast is another membranous system in the form of flattened, interconnected sacs called **thylakoids**. In some regions, thylakoids are stacked like poker chips; each stack is called a **granum** (plural, *grana*). The fluid outside the thylakoids is the **stroma**, which contains the chloroplast DNA and ribosomes as well as many enzymes. The membranes of the chloroplast divide the chloroplast space into three compartments: the intermembrane space, the stroma, and the thylakoid space. This compartmental organization enables the chloroplast to convert light energy to chemical energy

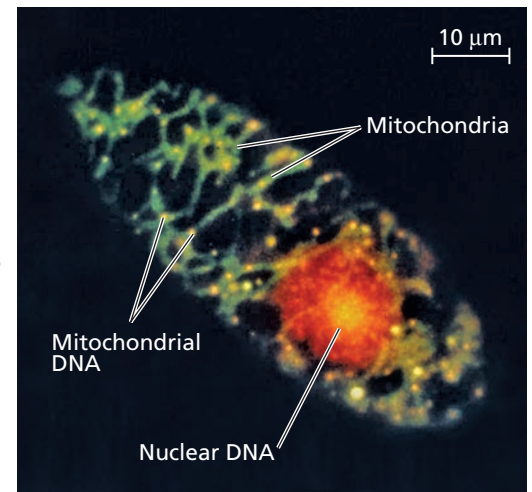
▼ **Figure 7.17 The mitochondrion, site of cellular respiration.** (a) The inner and outer membranes of the mitochondrion are evident in the drawing and electron micrograph (TEM). The cristae are infoldings of the inner membrane, which increase its surface area. The cutaway drawing shows the two compartments bounded

by the membranes: the intermembrane space and the mitochondrial matrix. Many respiratory enzymes are found in the inner membrane and the matrix. Free ribosomes are also present in the matrix. The circular DNA molecules are associated with the inner mitochondrial membrane. (b) The light micrograph shows an

entire unicellular eukaryote (*Euglena gracilis*) at a much lower magnification than the TEM. The mitochondrial matrix has been stained green. The mitochondria form a branched tubular network. The nuclear DNA is stained red; molecules of mitochondrial DNA appear as bright yellow spots.



(a) Diagram and TEM of mitochondrion



(b) Network of mitochondria in *Euglena* (LM)

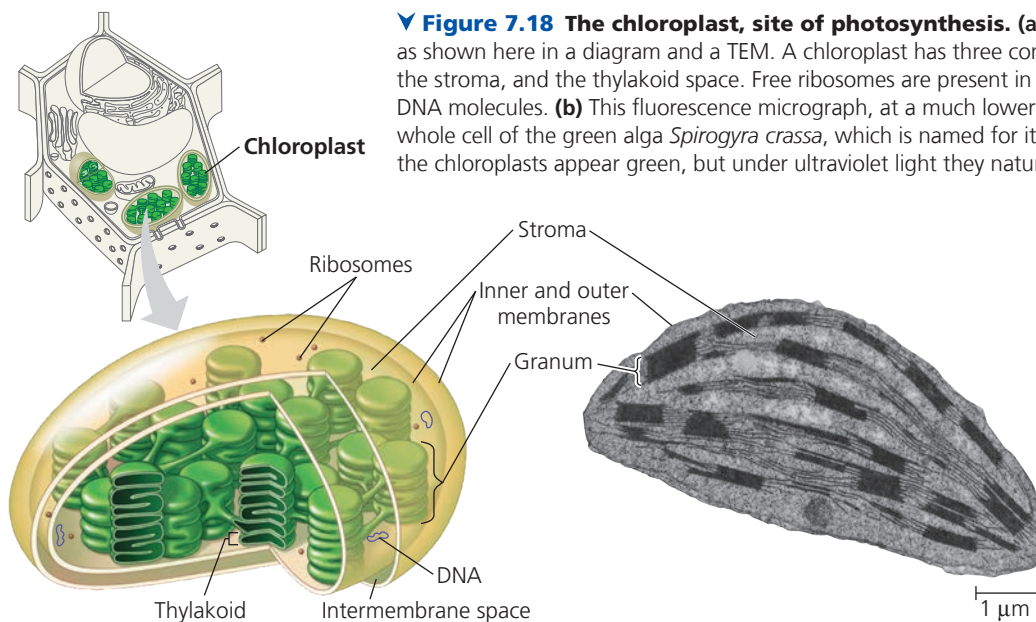
 **BioFlix® Animation: Mitochondria**

during photosynthesis. (You will learn more about photosynthesis in Chapter 11.)

As with mitochondria, the static and rigid appearance of chloroplasts in micrographs or schematic diagrams cannot accurately depict their dynamic behavior in the living cell. Their shape is changeable, and they grow and occasionally pinch in two, reproducing themselves. They are mobile and, with mitochondria and other organelles, move around the

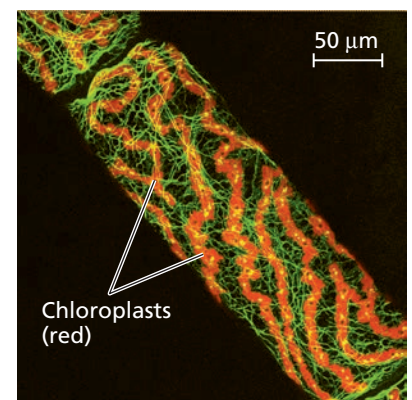
cell along tracks of the cytoskeleton, a structural network we will consider in Concept 7.6.

The chloroplast is a specialized member of a family of closely related plant organelles called **plastids**. One type of plastid, the *amyloplast*, is a colorless organelle that stores starch (amylose), particularly in roots and tubers. Another is the *chromoplast*, which has pigments that give fruits and flowers their orange and yellow hues.



(a) Diagram and TEM of chloroplast

▼ **Figure 7.18 The chloroplast, site of photosynthesis.** (a) Many plants have lens-shaped chloroplasts, as shown here in a diagram and a TEM. A chloroplast has three compartments: the intermembrane space, the stroma, and the thylakoid space. Free ribosomes are present in the stroma, as are copies of chloroplast DNA molecules. (b) This fluorescence micrograph, at a much lower magnification than the TEM, shows a whole cell of the green alga *Spirogyra crassa*, which is named for its spiral chloroplasts. Under natural light the chloroplasts appear green, but under ultraviolet light they naturally fluoresce red, as shown here.

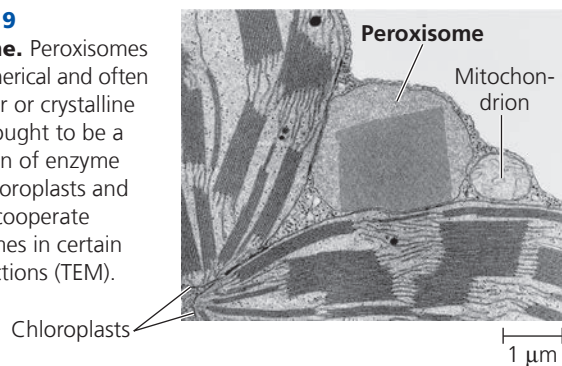


(b) Chloroplasts in an algal cell

 **BioFlix® Animation: Chloroplasts and Mitochondria**

► Figure 7.19

A peroxisome. Peroxisomes are roughly spherical and often have a granular or crystalline core that is thought to be a dense collection of enzyme molecules. Chloroplasts and mitochondria cooperate with peroxisomes in certain metabolic functions (TEM).



Peroxisomes: Oxidation

The **peroxisome** is a specialized metabolic compartment bounded by a single membrane (Figure 7.19). Peroxisomes contain enzymes that remove hydrogen atoms from various substrates and transfer them to oxygen (O_2), producing hydrogen peroxide (H_2O_2) as a by-product (from which the organelle derives its name). These reactions have many different functions. Some peroxisomes use oxygen to break fatty acids down into smaller molecules that are transported to mitochondria and used as fuel for cellular respiration. Peroxisomes in the liver detoxify alcohol and other harmful compounds by transferring hydrogen from the poisonous compounds to oxygen. The H_2O_2 formed by peroxisomes is itself toxic, but the organelle also contains an enzyme that converts H_2O_2 to water. This is an excellent example of how the cell's compartmental structure is crucial to its functions: The enzymes that produce H_2O_2 and those that dispose of this toxic compound are sequestered away from other cellular components that could be damaged.

Specialized peroxisomes called *glyoxysomes* are found in the fat-storing tissues of plant seeds. These organelles contain enzymes that initiate the conversion of fatty acids to sugar, which the emerging seedling uses as a source of energy and carbon until it can produce its own sugar by photosynthesis.

How peroxisomes are related to other organelles is still an open question. They grow larger by incorporating proteins made in the cytosol and ER, as well as lipids made in the ER and within the peroxisome itself. Peroxisomes may increase in number by splitting in two when they reach a certain size, sparking the suggestion of an endosymbiotic evolutionary origin, but others argue against this scenario. Discussion of this issue is ongoing.

CONCEPT CHECK 7.5

1. Describe two characteristics shared by chloroplasts and mitochondria. Consider both function and membrane structure.
2. Do plant cells have mitochondria? Explain.
3. **WHAT IF? ►** A classmate proposes that mitochondria and chloroplasts should be classified in the endomembrane system. Argue against the proposal.

For suggested answers, see Appendix A.

CONCEPT 7.6

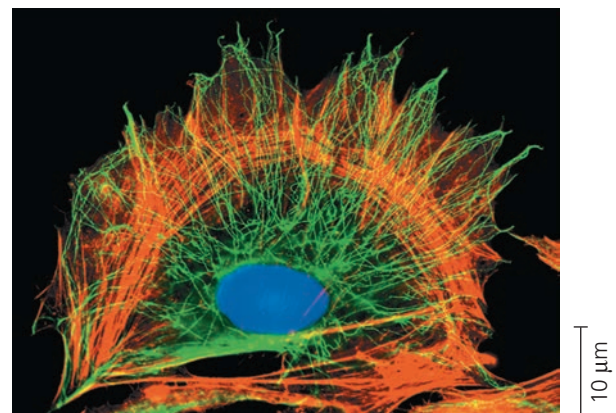
The cytoskeleton is a network of fibers that organizes structures and activities in the cell

In the early days of electron microscopy, biologists thought that the organelles of a eukaryotic cell floated freely in the cytosol. But improvements in both light microscopy and electron microscopy have revealed the **cytoskeleton**, a network of fibers extending throughout the cytoplasm (Figure 7.20). Bacterial cells also have fibers that form a type of cytoskeleton, constructed of proteins similar to eukaryotic ones, but here we will concentrate on eukaryotes. The eukaryotic cytoskeleton, which plays a major role in organizing the structures and activities of the cell, is composed of three types of molecular structures: microtubules, microfilaments, and intermediate filaments.

Roles of the Cytoskeleton: Support and Motility

The most obvious function of the cytoskeleton is to give mechanical support to the cell and maintain its shape. This is especially important for animal cells, which lack walls. The remarkable strength and resilience of the cytoskeleton as a whole are based on its architecture. Like a dome tent, the cytoskeleton is stabilized by a balance between opposing forces exerted by its elements. And just as the skeleton of an animal helps fix the positions of other body parts, the cytoskeleton provides anchorage for many organelles and even cytosolic enzyme molecules. The cytoskeleton is more dynamic than an animal skeleton, however. It can be quickly dismantled in one part of the cell and reassembled in a new location, changing the shape of the cell.

▼ **Figure 7.20 The cytoskeleton.** As shown in this fluorescence micrograph, the cytoskeleton extends throughout the cell. The cytoskeletal elements have been tagged with different fluorescent molecules: green for microtubules and reddish-orange for microfilaments. A third component of the cytoskeleton, intermediate filaments, is not evident here. (The blue color tags the DNA in the nucleus.)



BioFlix® Animation: Cytoskeleton

Some types of cell motility (movement) also involve the cytoskeleton. The term *cell motility* includes both changes in cell location and movements of cell parts. Cell motility generally requires interaction of the cytoskeleton with **motor proteins**. There are many such examples: Cytoskeletal elements and motor proteins work together with plasma membrane molecules to allow whole cells to move along fibers outside the cell. Inside the cell, vesicles and other organelles often use motor protein “feet” to “walk” to their destinations along a track provided by the cytoskeleton. For example, this is how vesicles containing neurotransmitter molecules migrate to the tips of axons, the long extensions of nerve cells that release these molecules as chemical signals to adjacent nerve cells (Figure 7.21). The cytoskeleton also manipulates the plasma membrane, bending it inward to form food vacuoles or other phagocytic vesicles.

 Interview with George Langford: Investigating how motor proteins move cargo

Components of the Cytoskeleton

Now let’s look more closely at the three main types of fibers that make up the cytoskeleton: *Microtubules* are the thickest of the three types; *microfilaments* (also called actin filaments) are the thinnest; and *intermediate filaments* are fibers with diameters in a middle range (Table 7.1).

▼ **Figure 7.21** Motor proteins and the cytoskeleton.

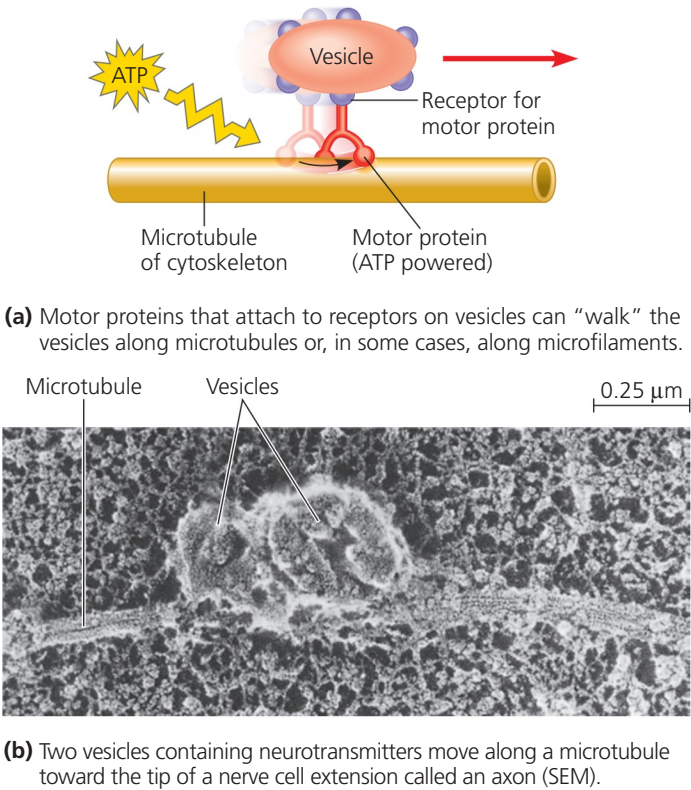
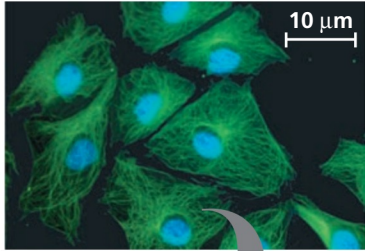
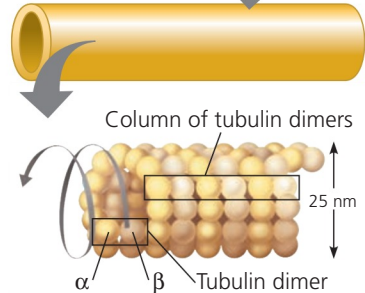
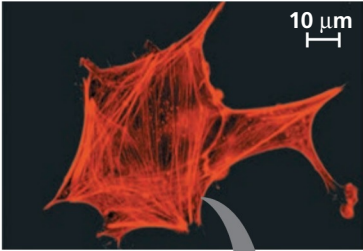
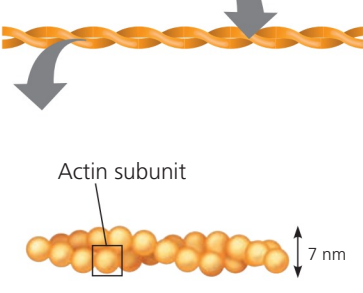
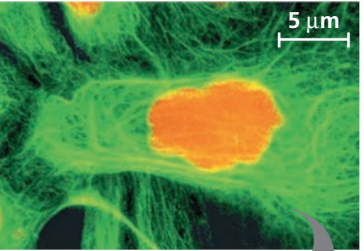
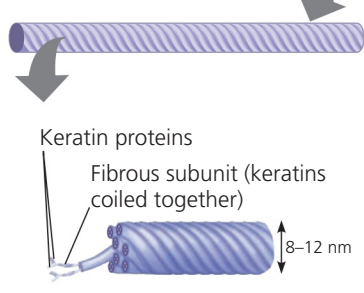


Table 7.1 The Structure and Function of the Cytoskeleton			
Property	Microtubules (Tubulin Polymers)	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes	Two intertwined strands of actin	Fibrous proteins coiled into cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, a dimer consisting of α -tubulin and β -tubulin	Actin	One of several different proteins (such as keratins)
Main functions	Maintenance of cell shape (compression-resisting “girders”); cell motility (as in cilia or flagella); chromosome movements in cell division; organelle movements	Maintenance of cell shape (tension-bearing elements); changes in cell shape; muscle contraction; cytoplasmic streaming in plant cells; cell motility (as in amoeboid movement); division of animal cells	Maintenance of cell shape (tension-bearing elements); anchorage of nucleus and certain other organelles; formation of nuclear lamina
Fluorescence micrographs of fibroblasts. Fibroblasts are a favorite cell type for cell biology studies because they spread out flat and their internal structures are easy to see. In each, the structure of interest has been tagged with fluorescent molecules. The DNA in the nucleus has also been tagged in the first micrograph (blue) and third micrograph (orange).	 	 	 

Microtubules

All eukaryotic cells have **microtubules**, hollow rods constructed from globular proteins called tubulins. Each tubulin protein is a *dimer*, a molecule made up of two subunits. A tubulin dimer consists of two slightly different polypeptides, α -tubulin and β -tubulin. Microtubules grow in length by adding tubulin dimers; they can also be disassembled and their tubulins used to build microtubules elsewhere in the cell. Because of the orientation of tubulin dimers, the two ends of a microtubule are slightly different. One end can accumulate or release tubulin dimers at a much higher rate than the other, thus growing and shrinking significantly during cellular activities. (This is called the “plus end,” not because it can only add tubulin proteins but because it’s the end where both “on” and “off” rates are much higher.)

Microtubules shape and support the cell and also serve as tracks along which organelles equipped with motor proteins can move. In addition to the example in Figure 7.21, microtubules guide vesicles from the ER to the Golgi apparatus and from the Golgi to the plasma membrane. Microtubules are also involved in the separation of chromosomes during cell division, as shown in Figure 12.7.

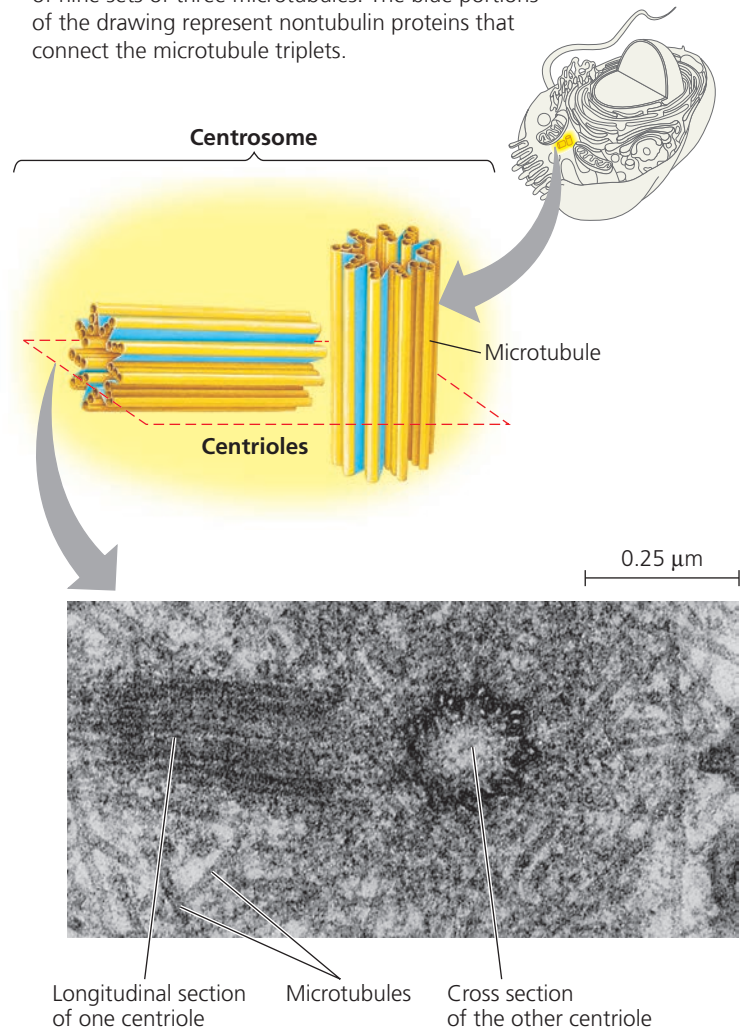
Centrosomes and Centrioles In animal cells, microtubules grow out from a **centrosome**, a region that is often located near the nucleus. These microtubules function as compression-resisting girders of the cytoskeleton. Within the centrosome is a pair of **centrioles**, each composed of nine sets of triplet microtubules arranged in a ring (**Figure 7.22**). Although centrosomes with centrioles may help organize microtubule assembly in animal cells, many other eukaryotic cells lack centrosomes with centrioles and instead organize microtubules by other means.

Cilia and Flagella In eukaryotes, a specialized arrangement of microtubules is responsible for the beating of **flagella** (singular, *flagellum*) and **cilia** (singular, *cilium*), microtubule-containing extensions that project from some cells. (The bacterial flagellum, shown in Figure 7.5, has a completely different structure.) Many unicellular eukaryotes are propelled through water by cilia or flagella that act as locomotor appendages, and the sperm of animals, algae, and some plants have flagella. When cilia or flagella extend from cells that are held in place as part of a tissue layer, they can move fluid over the surface of the tissue. For example, the ciliated lining of the trachea (windpipe) sweeps mucus containing trapped debris out of the lungs (see the EMs in Figure 7.3). In a woman’s reproductive tract, the cilia lining the oviducts help move an egg toward the uterus.

Motile cilia usually occur in large numbers on the cell surface. Flagella are usually limited to just one or a few per cell, and they are longer than cilia. Flagella and cilia differ in their

▼ Figure 7.22 Centrosome containing a pair of centrioles.

Most animal cells have a centrosome, a region near the nucleus where the cell’s microtubules are initiated. Within the centrosome is a pair of centrioles, each about 250 nm (0.25 μ m) in diameter. The two centrioles are at right angles to each other, and each is made up of nine sets of three microtubules. The blue portions of the drawing represent nontubulin proteins that connect the microtubule triplets.



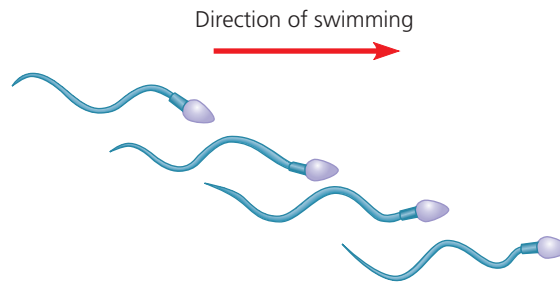
VISUAL SKILLS ► How many microtubules are in a centrosome? In the drawing, circle and label one microtubule and describe its structure. Circle and label a triplet.

beating patterns. A flagellum has an undulating motion like the tail of a fish. In contrast, cilia have alternating power and recovery strokes, much like the oars of a racing crew boat (**Figure 7.23**).

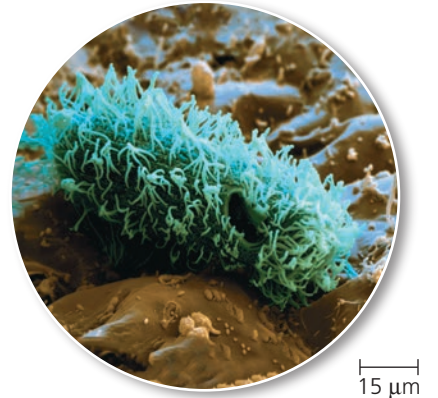
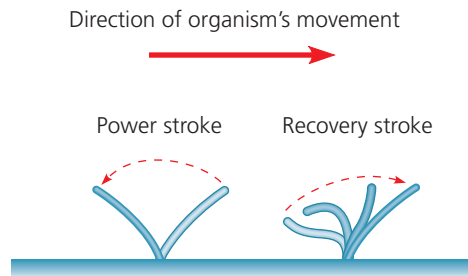
A cilium may also act as a signal-receiving “antenna” for the cell. Cilia that have this function are generally nonmotile, and there is only one per cell. (In fact, in vertebrate animals, it appears that almost all cells have such a cilium, which is called a *primary cilium*.) Membrane proteins on this kind of cilium transmit molecular signals from the cell’s environment to its interior, triggering signaling pathways that may lead to changes in the cell’s activities. Cilium-based signaling appears to be crucial to brain function and to embryonic development.

▼ **Figure 7.23** A comparison of the beating of flagella and motile cilia.

(a) Motion of flagella. A flagellum usually undulates, its snakelike motion driving a cell in the same direction as the axis of the flagellum. Propulsion of a human sperm cell is an example of flagellate locomotion (LM).



(b) Motion of cilia. Cilia have a back-and-forth motion. The rapid power stroke moves the cell in a direction perpendicular to the axis of the cilium. Then, during the slower recovery stroke, the cilium bends and sweeps sideways, closer to the cell surface. A dense nap of cilia, beating at a rate of about 40 to 60 strokes a second, covers this *Colpidium*, a freshwater protist (colorized SEM).



Video: Flagellum Movement in Swimming Sperm
Video: Flagellum Beating with ATP
Video: Paramecium Cilia

Though different in length, number per cell, and beating pattern, motile cilia and flagella share a common structure. Each motile cilium or flagellum has a group of microtubules sheathed in an extension of the plasma membrane (**Figure 7.24a**). Nine doublets of microtubules are arranged in a ring with two single microtubules in its center (**Figure 7.24b**). This arrangement, referred to as the “9 + 2” pattern, is found in nearly all eukaryotic flagella and motile cilia. (Nonmotile primary cilia have a “9 + 0” pattern, lacking the central pair of microtubules.) The microtubule assembly of a cilium or flagellum is anchored in the cell by a **basal body**, which is structurally very similar to a centriole, with microtubule triplets in a “9 + 0” pattern (**Figure 7.24c**). In fact, in many animals (including humans), the basal body of the fertilizing sperm’s flagellum enters the egg and becomes a centriole.

How does the microtubule assembly produce the bending movements of flagella and motile cilia? Bending involves large motor proteins called **dyneins** (red in the diagram in Figure 7.24) that are attached along each outer microtubule doublet. A typical dynein protein has two “feet” that “walk” along the microtubule of the adjacent doublet, using ATP for energy. One foot maintains contact, while the other releases and reattaches one step farther

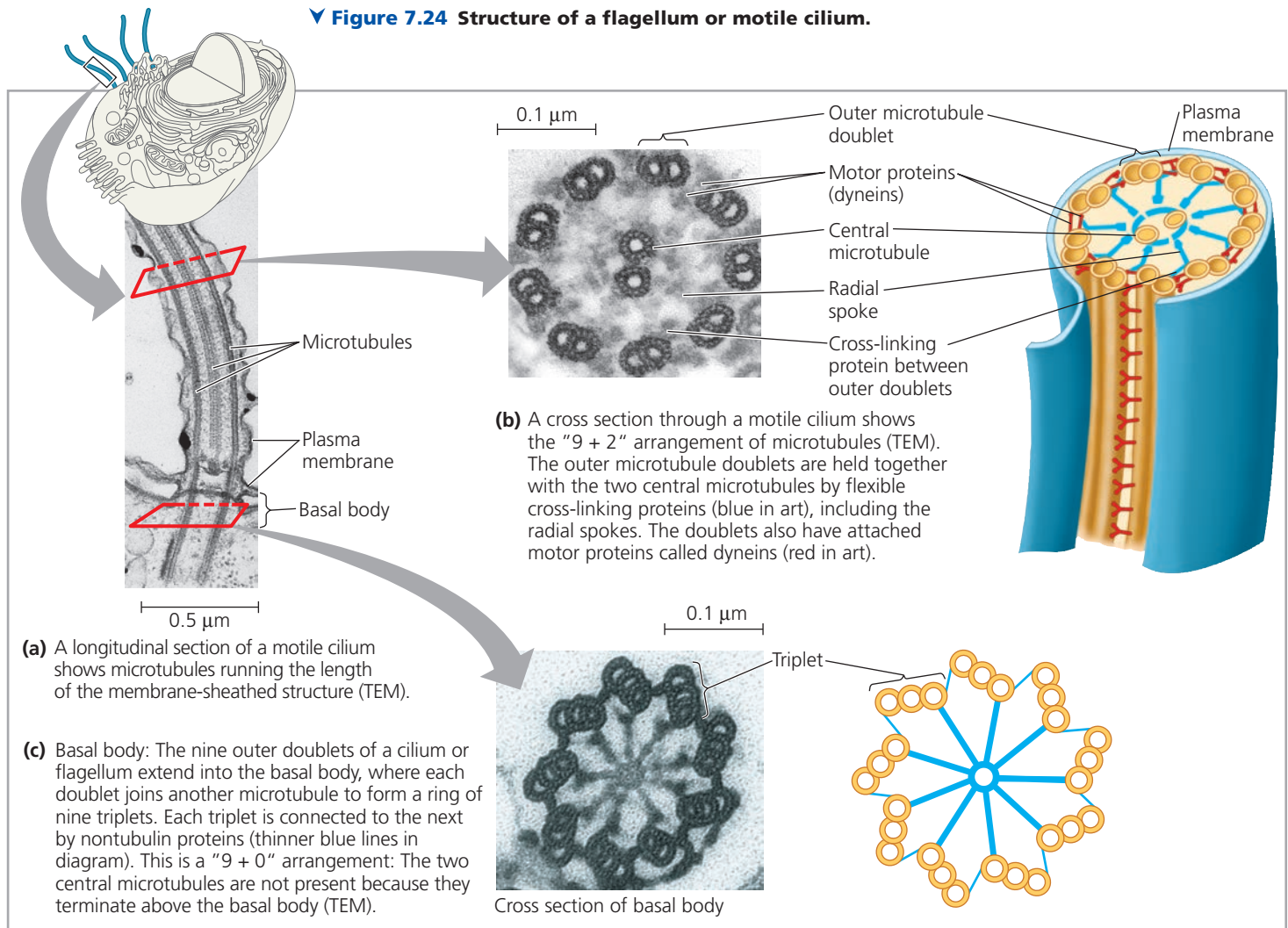
along the microtubule (see Figure 7.21). The outer doublets and two central microtubules are held together by flexible cross-linking proteins (blue in the diagram in Figure 7.24), and the walking movement is coordinated so that it happens on one side of the circle at a time. If the doublets were not held in place, the walking action would make them slide past each other. Instead, the movements of the dynein feet cause the microtubules—and the organelle as a whole—to bend.

Microfilaments (Actin Filaments)

Microfilaments are thin solid rods. They are also called actin filaments because they are built from molecules of **actin**, a globular protein. A microfilament is a twisted double chain of actin subunits (see Table 7.1). Besides occurring as linear filaments, microfilaments can form structural networks when certain proteins bind along the side of such a filament and allow a new filament to extend as a branch. Like microtubules, microfilaments seem to be present in all eukaryotic cells.

In contrast to the compression-resisting role of microtubules, the structural role of microfilaments in the cytoskeleton is to bear tension (pulling forces). A three-dimensional

▼ **Figure 7.24 Structure of a flagellum or motile cilium.**

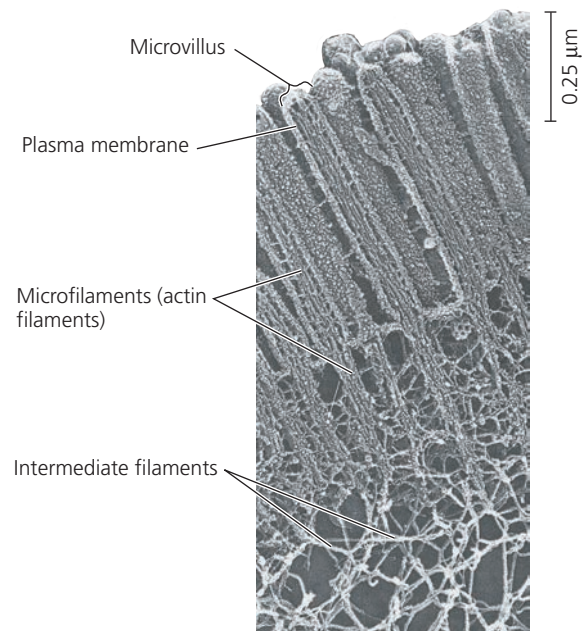


DRAW IT ► In (a) and (b), circle and label the central pair of microtubules. In (a), show where they terminate, and explain why they aren't seen in the cross section of the basal body in (c).

Animation: Cilia and Flagella

network formed by microfilaments just inside the plasma membrane (*cortical microfilaments*) helps support the cell's shape (see Figure 7.8). This network gives the outer cytoplasmic layer of a cell, called the **cortex**, the semisolid consistency of a gel, in contrast with the more fluid state of the interior cytoplasm. In some kinds of animal cells, such as nutrient-absorbing intestinal cells, bundles of microfilaments make up the core of microvilli, delicate projections that increase the cell's surface area (**Figure 7.25**).

Microfilaments are well known for their role in cell motility. Thousands of actin filaments and thicker filaments



► **Figure 7.25 A structural role of microfilaments.** The surface area of this nutrient-absorbing intestinal cell is increased by its many microvilli (singular, *microvillus*), cellular extensions reinforced by bundles of microfilaments. These actin filaments are anchored to a network of intermediate filaments (TEM).

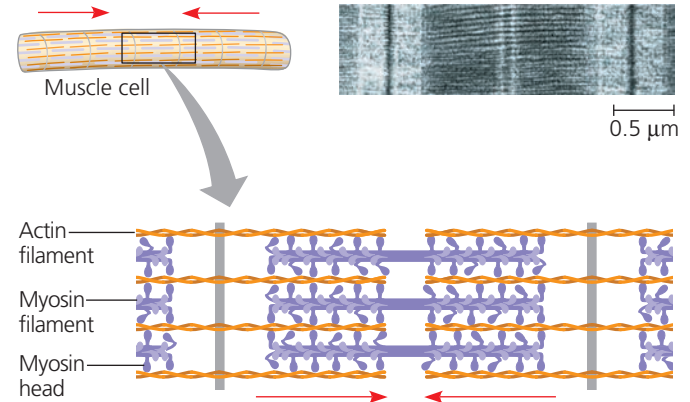
made of a protein called **myosin** interact to cause contraction of muscle cells (**Figure 7.26a**); muscle contraction is described in detail in Concept 50.5. In the unicellular eukaryote *Amoeba* and some of our white blood cells, localized contractions brought about by actin and myosin are involved in the amoeboid (crawling) movement of the cells. The cell crawls along a surface by extending cellular extensions called **pseudopodia** (from the Greek *pseudes*, false, and *pod*, foot) and moving toward them (**Figure 7.26b**). In plant cells, actin-protein interactions contribute to **cytoplasmic streaming**, a circular flow of cytoplasm within cells (**Figure 7.26c**). This movement, which is especially common in large plant cells, speeds the movement of organelles and the distribution of materials within the cell.

Intermediate Filaments

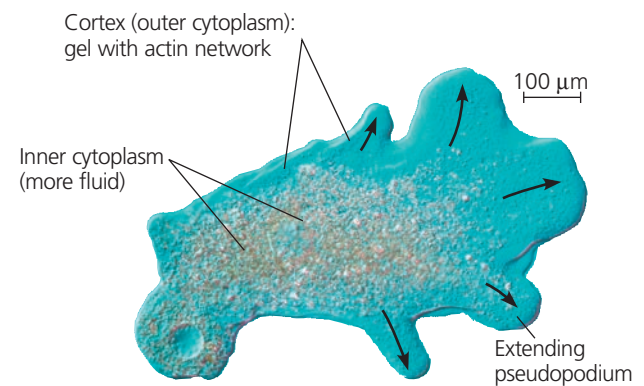
Intermediate filaments are named for their diameter, which is larger than the diameter of microfilaments but smaller than that of microtubules (see Table 7.1). While microtubules and microfilaments are found in all eukaryotic cells, intermediate filaments are only found in the cells of some animals, including vertebrates. Specialized for bearing tension (like microfilaments), intermediate filaments are a diverse class of cytoskeletal elements. Each type is constructed from a particular molecular subunit belonging to a family of proteins whose members include the keratins. Microtubules and microfilaments, in contrast, are consistent in diameter and composition in all eukaryotic cells.

Intermediate filaments are more permanent fixtures of cells than are microfilaments and microtubules, which are often disassembled and reassembled in various parts of a cell. Even after cells die, intermediate filament networks often persist; for example, the outer layer of our skin consists of dead skin cells full of keratin filaments. Chemical treatments that remove microfilaments and microtubules from the cytoplasm of living cells leave a web of intermediate filaments that retains its original shape. Such experiments suggest that intermediate filaments are especially sturdy and that they play an important role in reinforcing the shape of a cell and fixing the position of certain organelles. For instance, the nucleus typically sits within a cage made of intermediate filaments, fixed in location by branches of the filaments that extend into the cytoplasm. Other intermediate filaments make up the nuclear lamina, which lines the interior of the nuclear envelope (see Figure 7.9). By supporting a cell's shape, intermediate filaments help the cell carry out its specific function. For example, the network of intermediate filaments shown in Figure 7.25 anchors the microfilaments supporting the intestinal microvilli. Thus, the various kinds of intermediate filaments may function together as the permanent framework of the entire cell.

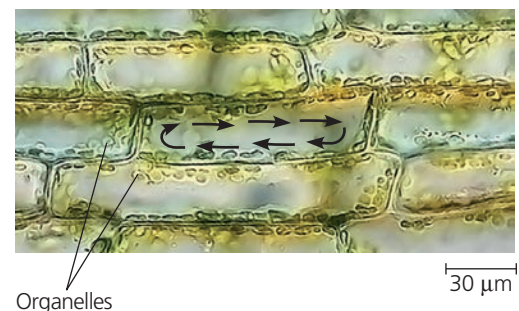
Figure 7.26 Microfilaments and motility. In these three examples, interactions between actin filaments and motor proteins bring about cell movement.



(a) Myosin motors in muscle cell contraction. The “walking” of myosin projections (the so-called heads) drives the parallel myosin and actin filaments past each other so that the actin filaments approach each other in the middle (red arrows). This shortens the muscle cell. Muscle contraction involves the shortening of many muscle cells at the same time (TEM).



(b) Amoeboid movement. Interaction of actin filaments with myosin causes contraction of the cell, pulling the cell's trailing end (at left) forward (to the right) (LM).



(c) Cytoplasmic streaming in plant cells. A layer of cytoplasm cycles around the cell, moving over tracks of actin filaments. Myosin motors attached to some organelles drive the streaming by interacting with the actin (LM).



BioFlix® Animation: Actin and Myosin in Muscle Contraction
Video: Amoeba Pseudopodia
Video: Cytoplasmic Streaming

CONCEPT CHECK 7.6

1. Describe how cilia and flagella bend.
2. **WHAT IF? >** Males afflicted with Kartagener's syndrome are sterile because of immotile sperm, and they tend to suffer from lung infections. This disorder has a genetic basis. Suggest what the underlying defect might be.

For suggested answers, see Appendix A.

CONCEPT 7.7

Extracellular components and connections between cells help coordinate cellular activities

Having crisscrossed the cell to explore its interior components, we complete our tour of the cell by returning to the surface of this microscopic world, where there are additional structures with important functions. The plasma membrane is usually regarded as the boundary of the living cell, but most cells synthesize and secrete materials extracellularly (to the outside of the cell). Although these materials and the structures they form are outside the cell, their study is important to cell biology because they are involved in a great many essential cellular functions.

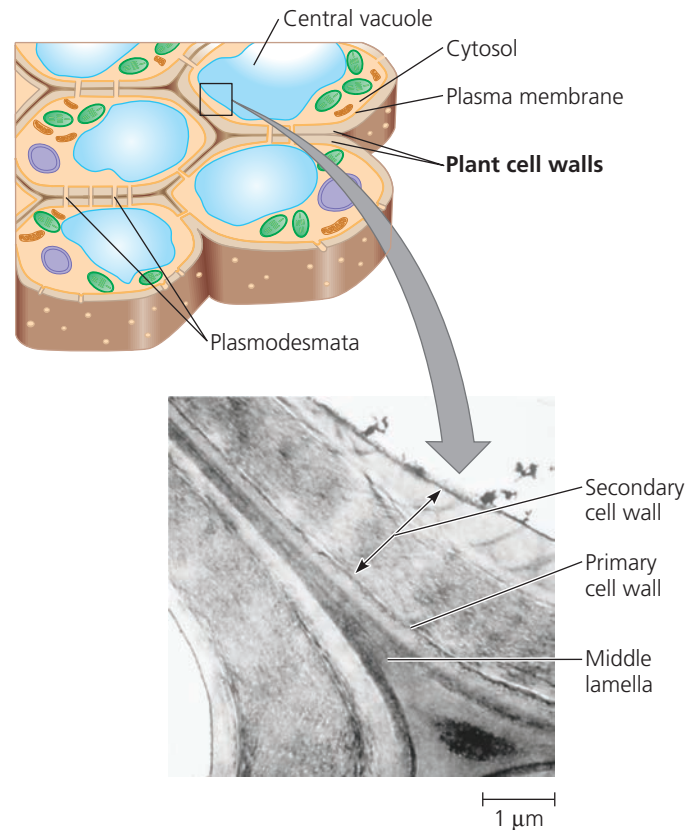
Cell Walls of Plants

The **cell wall** is an extracellular structure of plant cells (Figure 7.27). This is one of the features that distinguishes plant cells from animal cells. The wall protects the plant cell, maintains its shape, and prevents excessive uptake of water. On the level of the whole plant, the strong walls of specialized cells hold the plant up against the force of gravity. Prokaryotes, fungi, and some unicellular eukaryotes also have cell walls, as you saw in Figures 7.5 and 7.8, but we will postpone discussion of them until Unit Five.

Plant cell walls are much thicker than the plasma membrane, ranging from 0.1 μm to several micrometers. The exact chemical composition of the wall varies from species to species and even from one cell type to another in the same plant, but the basic design of the wall is consistent. Microfibrils made of the polysaccharide cellulose (see Figure 5.6) are synthesized by an enzyme called cellulose synthase and secreted to the extracellular space, where they become embedded in a matrix of other polysaccharides and proteins. This combination of materials, strong fibers in a "ground substance" (matrix), is the same basic architectural design found in steel-reinforced concrete and in fiberglass.

A young plant cell first secretes a relatively thin and flexible wall called the **primary cell wall** (see the micrograph in Figure 7.27). Between primary walls of adjacent cells is the **middle lamella**, a thin layer rich in sticky polysaccharides called pectins. The middle lamella glues adjacent cells together. (Pectin is used in cooking as a thickening agent in

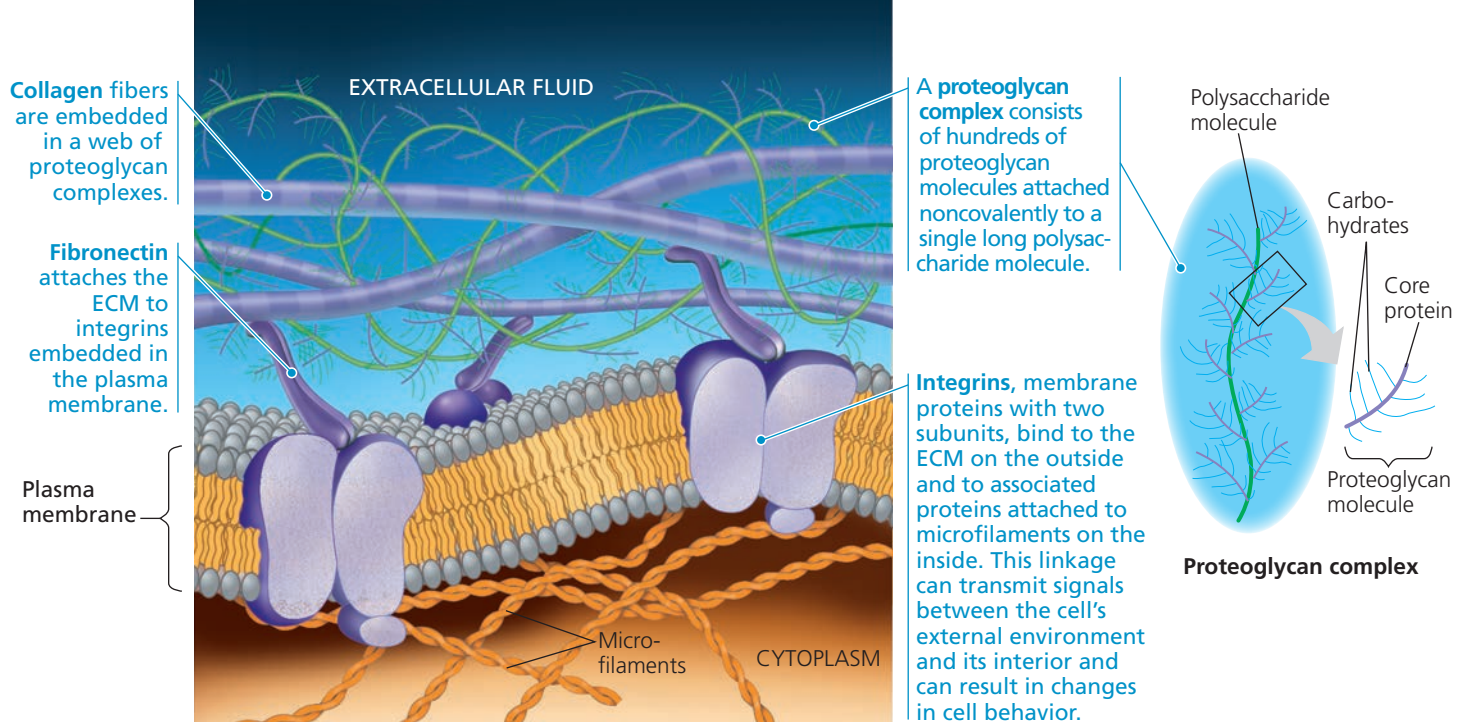
▼ **Figure 7.27 Plant cell walls.** The drawing shows several cells, each with a cell wall, large vacuole, a nucleus, and several chloroplasts and mitochondria. The TEM shows the cell walls where two cells come together. The multilayered partition between plant cells consists of adjoining walls individually secreted by the cells.



jams and jellies.) When the cell matures and stops growing, it strengthens its wall. Some plant cells do this simply by secreting hardening substances into the primary wall. Other cells add a **secondary cell wall** between the plasma membrane and the primary wall. The secondary wall, often deposited in several laminated layers, has a strong and durable matrix that affords the cell protection and support. Wood, for example, consists mainly of secondary walls. Plant cell walls are usually perforated by channels between adjacent cells called plasmodesmata, which will be discussed shortly.

The Extracellular Matrix (ECM) of Animal Cells

Although animal cells lack walls akin to those of plant cells, they do have an elaborate **extracellular matrix (ECM)**. The main ingredients of the ECM are glycoproteins and other carbohydrate-containing molecules secreted by the cells. (Recall that glycoproteins are proteins with covalently bonded carbohydrates, usually short chains of sugars.) The most abundant glycoprotein in the ECM of most animal cells is **collagen**, which forms strong fibers outside the cells (see Figure 5.18). In fact, collagen accounts for about 40% of the total protein in the human body. The collagen fibers are embedded in a network woven out of **proteoglycans**



▲ Figure 7.28 Extracellular matrix (ECM) of an animal cell. The molecular composition and structure of the ECM vary from one cell type to another. In this example, three different types of ECM molecules are present: collagen, fibronectin, and proteoglycans.

secreted by cells (**Figure 7.28**). A proteoglycan molecule consists of a small core protein with many carbohydrate chains covalently attached, so that it may be up to 95% carbohydrate. Large proteoglycan complexes can form when hundreds of proteoglycan molecules become noncovalently attached to a single long polysaccharide molecule, as shown in **Figure 7.28**. Some cells are attached to the ECM by ECM glycoproteins such as **fibronectin**. Fibronectin and other ECM proteins bind to cell-surface receptor proteins called **integrins** that are built into the plasma membrane. Integrins span the membrane and bind on their cytoplasmic side to associated proteins attached to microfilaments of the cytoskeleton. The name *integrin* is based on the word *integrate*: Integrins are in a position to transmit signals between the ECM and the cytoskeleton and thus to integrate changes occurring outside and inside the cell.

Current research on fibronectin, other ECM molecules, and integrins reveals the influential role of the ECM in the lives of cells. By communicating with a cell through integrins, the ECM can regulate a cell's behavior. For example, some cells in a developing embryo migrate along specific pathways by matching the orientation of their microfilaments to the "grain" of fibers in the extracellular matrix. Researchers have also learned that the extracellular matrix around a cell can influence the activity of genes in the nucleus. Information about the ECM probably reaches the nucleus by a combination of mechanical and chemical signaling pathways. Mechanical signaling involves fibronectin, integrins, and microfilaments of the cytoskeleton. Changes in the cytoskeleton may in turn trigger signaling pathways inside the cell, leading to changes in the set

of proteins being made by the cell and therefore changes in the cell's function. In this way, the extracellular matrix of a particular tissue may help coordinate the behavior of all the cells of that tissue. Direct connections between cells also function in this coordination, as we discuss next.

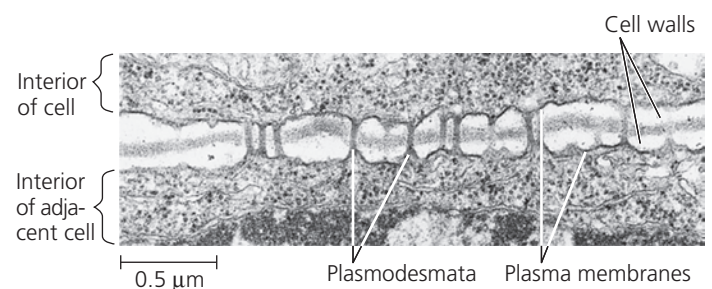
Cell Junctions

Cells in an animal or plant are organized into tissues, organs, and organ systems. Neighboring cells often adhere, interact, and communicate via sites of direct physical contact.

Plasmodesmata in Plant Cells

It might seem that the nonliving cell walls of plants would isolate plant cells from one another. But in fact, as shown in **Figure 7.29**, cell walls are perforated with **plasmodesmata** (singular, *plasmodesma*; from the Greek *desma*, bond), channels that connect cells. Cytosol passing through the

▼ Figure 7.29 Plasmodesmata between plant cells. The cytoplasm of one plant cell is continuous with the cytoplasm of its neighbors via plasmodesmata, cytoplasmic channels through the cell walls (TEM).



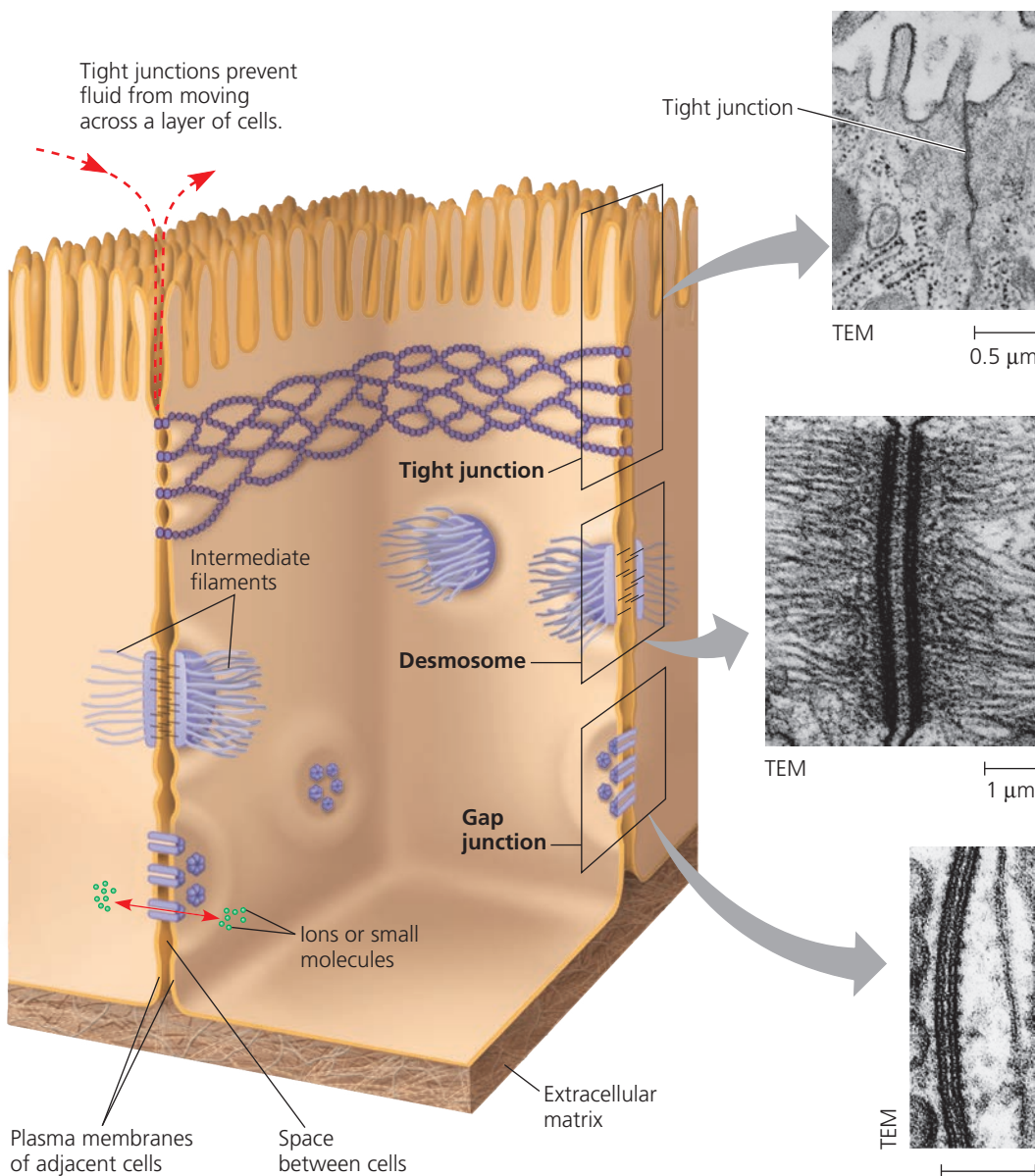
plasmodesmata joins the internal chemical environments of adjacent cells. These connections unify most of the plant into one living continuum. The plasma membranes of adjacent cells line the channel of each plasmodesma and thus are continuous. Water and small solutes can pass freely from cell to cell, and several experiments have shown that in some circumstances, certain proteins and RNA molecules can do this as well (see Concept 36.6). The macromolecules transported to neighboring cells appear to reach the plasmodesmata by moving along fibers of the cytoskeleton.

Tight Junctions, Desmosomes, and Gap Junctions in Animal Cells

In animals, there are three main types of cell junctions: *tight junctions*, *desmosomes*, and *gap junctions*. (Gap junctions are most like the plasmodesmata of plants, although gap junction pores are not lined with membrane.) All three types of cell junctions are especially common in epithelial tissue, which lines the external and internal surfaces of the body.

Figure 7.30 uses epithelial cells of the intestinal lining to illustrate these junctions.

▼ **Figure 7.30 Exploring Cell Junctions in Animal Tissues**



Tight Junctions

At **tight junctions**, the plasma membranes of neighboring cells are very tightly pressed against each other, bound together by specific proteins. Forming continuous seals around the cells, tight junctions establish a barrier that prevents leakage of extracellular fluid across a layer of epithelial cells (see red dashed arrow). For example, tight junctions between skin cells make us watertight.

Desmosomes

Desmosomes (one type of *anchoring junction*) function like rivets, fastening cells together into strong sheets. Intermediate filaments made of sturdy keratin proteins anchor desmosomes in the cytoplasm. Desmosomes attach muscle cells to each other in a muscle. Some “muscle tears” involve the rupture of desmosomes.

Gap Junctions

Gap junctions (also called *communicating junctions*) provide cytoplasmic channels from one cell to an adjacent cell and in this way are similar in their function to the plasmodesmata in plants. Gap junctions consist of membrane proteins that surround a pore through which ions, sugars, amino acids, and other small molecules may pass. Gap junctions are necessary for communication between cells in many types of tissues, such as heart muscle, and in animal embryos.

CONCEPT CHECK 7.7

1. In what way are the cells of plants and animals structurally different from single-celled eukaryotes?
2. **WHAT IF? >** Nonliving cell walls isolate plant cells from one another. Still, most of the plant can be considered to be one living continuum. Explain.
3. **MAKE CONNECTIONS >** The polypeptide chain that makes up a tight junction weaves back and forth through the membrane four times, with two extracellular loops, and one loop plus short C-terminal and N-terminal tails in the cytoplasm. Looking at Figure 5.14, what would you predict about the amino acid sequence of the tight junction protein?

For suggested answers, see Appendix A.

CONCEPT 7.8

A cell is greater than the sum of its parts

From our panoramic view of the cell's compartmental organization to our close-up inspection of each organelle's architecture, this tour of the cell has provided many opportunities to correlate structure with function. (This would be a good time to review cell structure by returning to Figure 7.8.)

 **BioFlix® Animation: Tour of an Animal Cell**
BioFlix® Animation: Tour of a Plant Cell

Even as we dissect the cell, remember that none of its components works alone. As an example of cellular integration, consider the microscopic scene in **Figure 7.31**. The large cell is a macrophage (see Figure 7.13a). It helps defend the mammalian body against infections by ingesting bacteria (the smaller

cells) into phagocytic vesicles. The macrophage crawls along a surface and reaches out to the bacteria with thin pseudopodia (specifically, filopodia). Actin filaments interact with other elements of the cytoskeleton in these movements. After the macrophage engulfs the bacteria, they are destroyed by lysosomes produced by the elaborate endomembrane system. The digestive enzymes of the lysosomes and the proteins of the cytoskeleton are all made by ribosomes. And the synthesis of these proteins is programmed by genetic messages dispatched from the DNA in the nucleus. All these processes require energy, which mitochondria supply in the form of ATP.

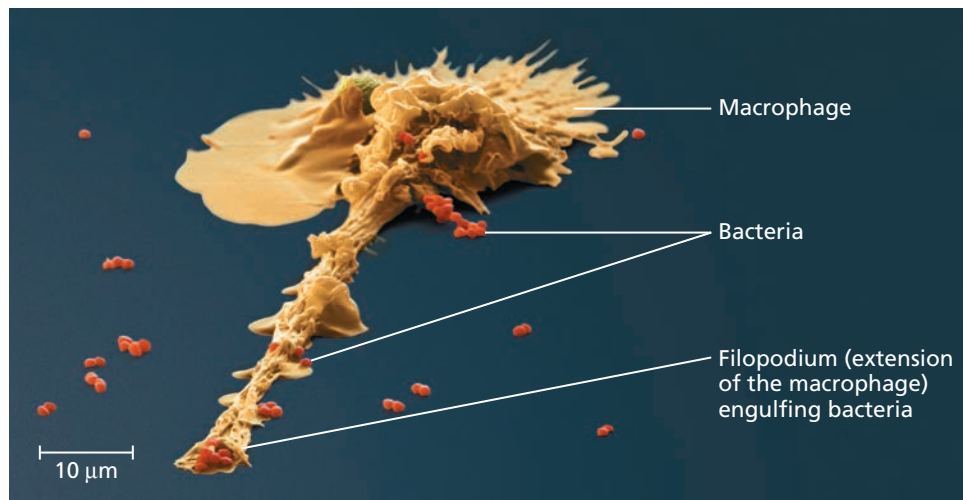
Cellular functions arise from cellular order: The cell is a living unit greater than the sum of its parts. The cell in Figure 7.31 is a good example of integration of cellular processes, seen from the outside. But what about the internal organization of a cell? As you proceed in your study of biology to consider different cellular processes, it will be helpful to try to visualize the architecture and furnishings inside a cell. **Figure 7.32** is designed to help you get a sense of the relative sizes and organization of important biological molecules and macromolecules, along with cellular structures and organelles. As you study this figure, see if you can shrink yourself down to the size of a protein and contemplate your surroundings.

CONCEPT CHECK 7.8

1. *Colpidium colpoda* is a unicellular eukaryote that lives in freshwater, eats bacteria, and moves by cilia (see Figure 7.23b). Describe how the parts of this cell work together in the functioning of *C. colpoda*, including as many organelles and other cell structures as you can.

For suggested answers, see Appendix A.

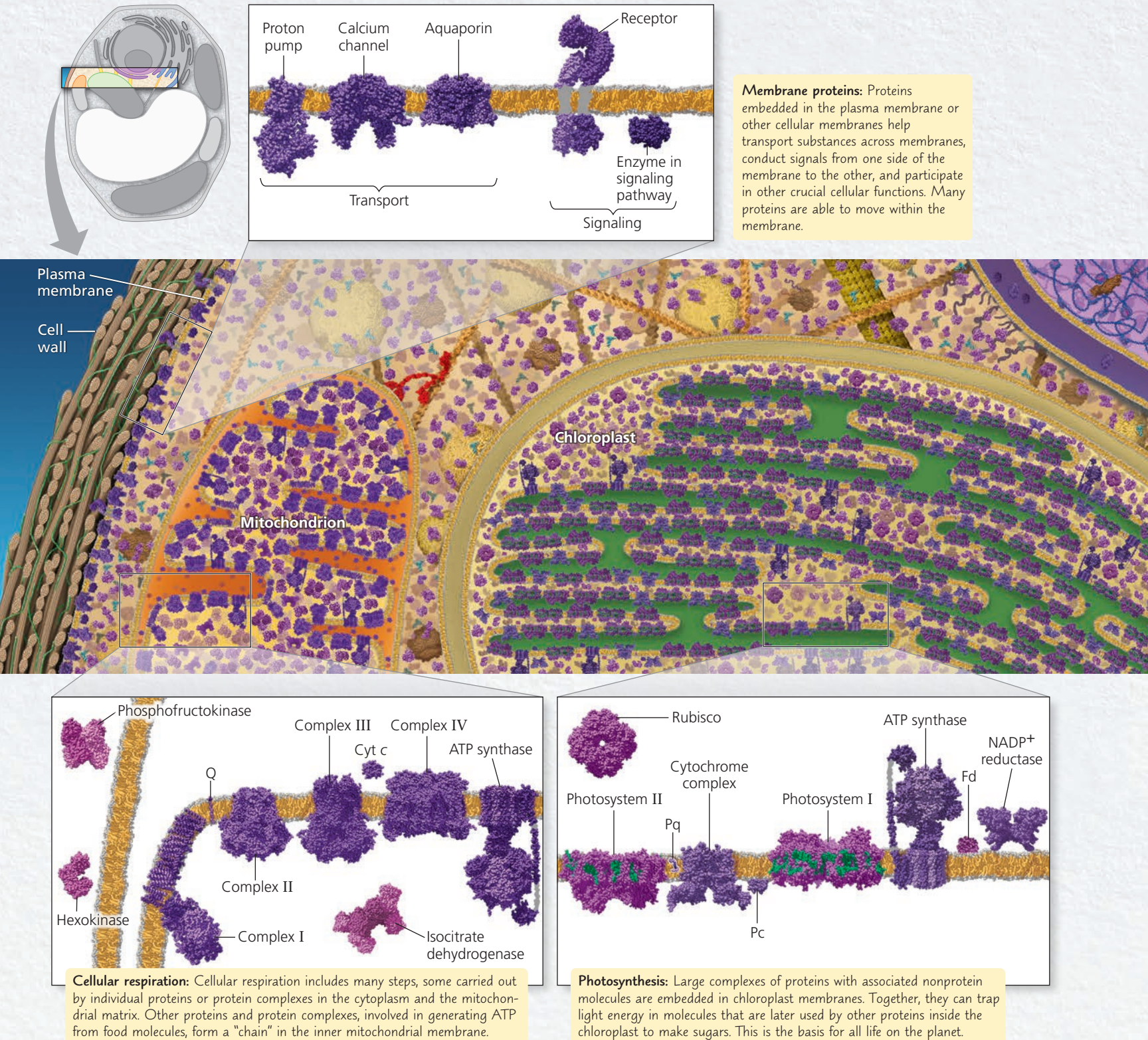
▼ **Figure 7.31 The emergence of cellular functions.** The ability of this macrophage (brown) to recognize, apprehend, and destroy *Staphylococcus* bacteria (orange) is a coordinated activity of the whole cell. Its cytoskeleton, lysosomes, and plasma membrane are among the components that function in phagocytosis (colorized SEM).



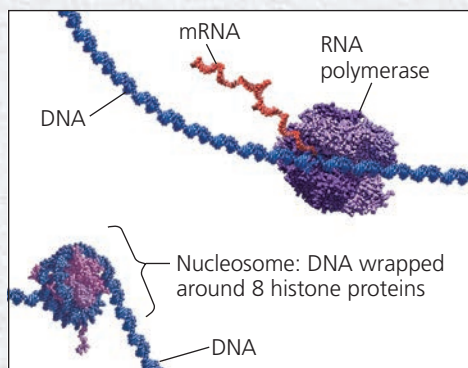
 **Animation: Review of Animal Cell Structure and Function**

▼ Figure 7.32 Visualizing the Scale of the Molecular Machinery in a Cell

A slice of a plant cell's interior is illustrated in the center panel, with all structures and molecules drawn to scale. Selected molecules and structures are shown above and below, all enlarged by the same factor so you can compare their sizes. All protein and nucleic acid structures are based on data from the Protein Data Bank; regions whose structure has not been determined are shown in gray.

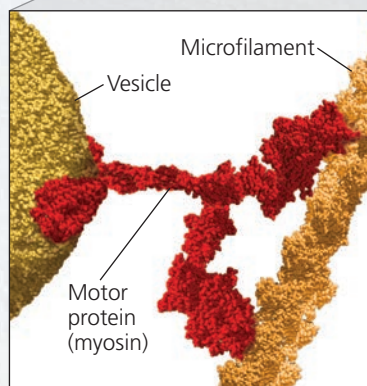
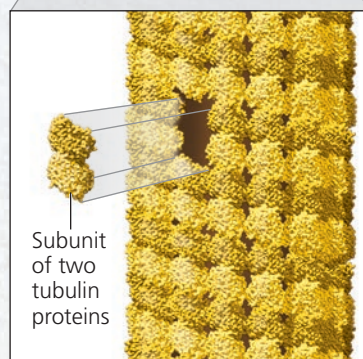
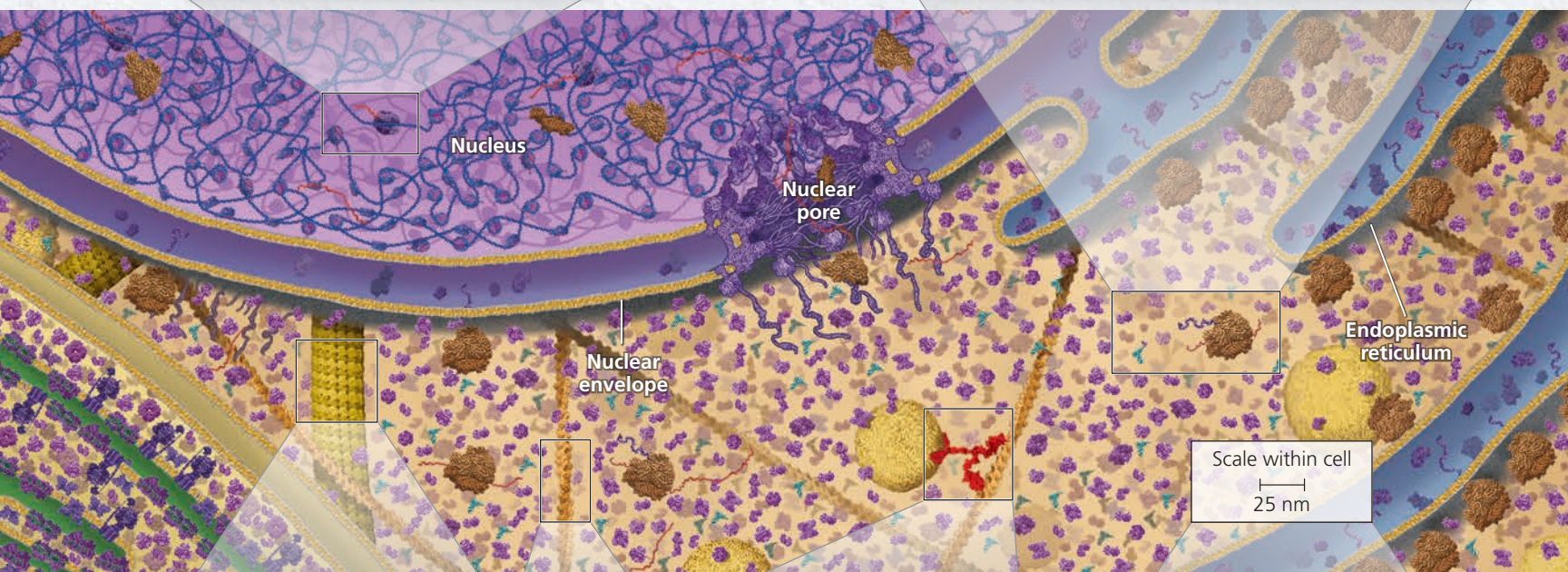
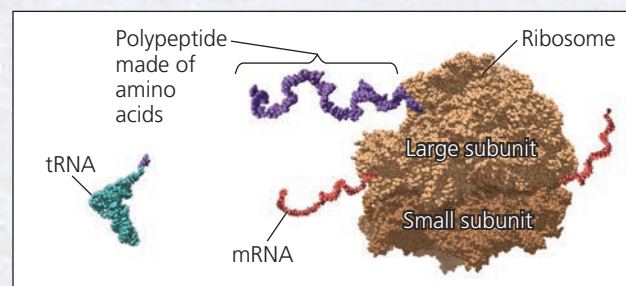


Transcription: In the nucleus, the information contained in a DNA sequence is transferred to messenger RNA (mRNA) by an enzyme called RNA polymerase. After their synthesis, mRNA molecules leave the nucleus via nuclear pores.



Nuclear pore: The nuclear pore complex regulates molecular traffic in and out of the nucleus, which is bounded by a double membrane. Among the largest structures that pass through the pore are the ribosomal subunits, which are built in the nucleus.

Translation: In the cytoplasm, the information in mRNA is used to assemble a polypeptide with a specific sequence of amino acids. Both transfer RNA (tRNA) molecules and a ribosome play a role. The eukaryotic ribosome, which includes a large subunit and a small subunit, is a colossal complex composed of four large ribosomal RNA (rRNA) molecules and more than 80 proteins. Through transcription and translation, the nucleotide sequence of DNA determines the amino acid sequence of a polypeptide, via the intermediary mRNA.



Cytoskeleton: Cytoskeletal structures are polymers of protein subunits. Microtubules are hollow structural rods made of tubulin protein subunits, while microfilaments are cables that have two chains of actin proteins wound around each other.

Motor proteins: Responsible for transport of vesicles and movement of organelles within the cell. This requires energy, often provided by ATP hydrolysis.

Scale within cell
25 nm

25 nm
Scale of enlarged structures

- 1 List the following structures from largest to smallest: proton pump, nuclear pore, Cyt c, ribosome.
- 2 Considering the structures of a nucleosome and of RNA polymerase, speculate about what must happen before RNA polymerase can transcribe the DNA wrapped around the histone proteins of a nucleosome.
- 3 Find another myosin motor protein walking on a microfilament in this figure. What organelle is being moved by that myosin protein?



Instructors: Additional questions related to this Visualizing Figure can be assigned in MasteringBiology.

7 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 7.1

Biologists use microscopes and biochemistry to study cells (pp. 164–167)

- Improvements in microscopy that affect the parameters of magnification, resolution, and contrast have catalyzed progress in the study of cell structure. **Light microscopy** (LM) and **electron microscopy** (EM), as well as other types, remain important tools.
- Cell biologists can obtain pellets enriched in particular cellular components by centrifuging disrupted cells at sequential speeds, a process known as **cell fractionation**.

? How do microscopy and biochemistry complement each other to reveal cell structure and function?



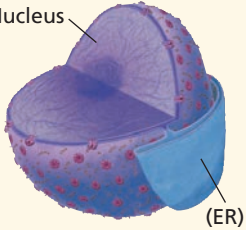
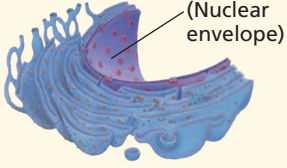
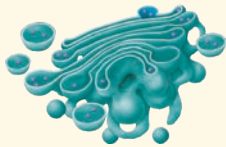
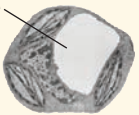
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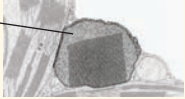
CONCEPT 7.2

Eukaryotic cells have internal membranes that compartmentalize their functions (pp. 167–172)

- All cells are bounded by a **plasma membrane**.
- Prokaryotic cells** lack nuclei and other membrane-enclosed **organelles**, while **eukaryotic cells** have internal membranes that compartmentalize cellular functions.
- The surface-to-volume ratio is an important parameter affecting cell size and shape.
- Plant and animal cells have most of the same organelles: a nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondria. Chloroplasts are present only in cells of photosynthetic eukaryotes.

? Explain how the compartmental organization of a eukaryotic cell contributes to its biochemical functioning.

	Cell Component	Structure	Function
CONCEPT 7.3 The eukaryotic cell's genetic instructions are housed in the nucleus and carried out by the ribosomes (pp. 172–174) ? Describe the relationship between the nucleus and ribosomes.	Nucleus 	Surrounded by nuclear envelope (double membrane) perforated by nuclear pores; nuclear envelope continuous with endoplasmic reticulum (ER)	Houses chromosomes, which are made of chromatin (DNA and proteins); contains nucleoli, where ribosomal subunits are made; pores regulate entry and exit of materials
	Ribosome 	Two subunits made of ribosomal RNAs and proteins; can be free in cytosol or bound to ER	Protein synthesis
CONCEPT 7.4 The endomembrane system regulates protein traffic and performs metabolic functions (pp. 174–178) ? Describe the key role played by transport vesicles in the endomembrane system.	Endoplasmic reticulum (ER) 	Extensive network of membrane-bounded tubules and sacs; membrane separates lumen from cytosol; continuous with nuclear envelope	Smooth ER: synthesis of lipids, metabolism of carbohydrates, Ca ²⁺ storage, detoxification of drugs and poisons Rough ER: aids in synthesis of secretory and other proteins on bound ribosomes; adds carbohydrates to proteins to make glycoproteins; produces new membrane
	Golgi apparatus 	Stacks of flattened membranous sacs; has polarity (<i>cis</i> and <i>trans</i> faces)	Modification of proteins, carbohydrates on proteins, and phospholipids; synthesis of many polysaccharides; sorting of Golgi products, which are then released in vesicles
	Lysosome 	Membranous sac of hydrolytic enzymes (in animal cells)	Breakdown of ingested substances, cell macromolecules, and damaged organelles for recycling
	Vacuole 	Large membrane-bounded vesicle	Digestion, storage, waste disposal, water balance, cell growth, and protection

	Cell Component	Structure	Function
CONCEPT 7.5 Mitochondria and chloroplasts change energy from one form to another (pp. 179–182)  What does the endosymbiont theory propose as the origin for mitochondria and chloroplasts? Explain.	Mitochondrion 	Bounded by double membrane; inner membrane has infoldings	Cellular respiration
	Chloroplast 	Typically two membranes around fluid stroma, which contains thylakoids stacked into grana	Photosynthesis (chloroplasts are in cells of photosynthetic eukaryotes, including plants)
	Peroxisome 	Specialized metabolic compartment bounded by a single membrane	Contains enzymes that transfer H atoms from substrates to oxygen, producing H ₂ O ₂ (hydrogen peroxide), which is converted to H ₂ O.

CONCEPT 7.6

The cytoskeleton is a network of fibers that organizes structures and activities in the cell (pp. 182–188)

- The **cytoskeleton** functions in structural support for the cell and in motility and signal transmission.
- Microtubules** shape the cell, guide organelle movement, and separate chromosomes in dividing cells. **Cilia** and **flagella** are motile appendages containing microtubules. Primary cilia also play sensory and signaling roles. **Microfilaments** are thin rods that function in muscle contraction, amoeboid movement, **cytoplasmic streaming**, and support of microvilli. **Intermediate filaments** support cell shape and fix organelles in place.

 Describe the role of motor proteins inside the eukaryotic cell and in whole-cell movement.

CONCEPT 7.7

Extracellular components and connections between cells help coordinate cellular activities (pp. 188–191)

- Plant **cell walls** are made of cellulose fibers embedded in other polysaccharides and proteins.
- Animal cells secrete glycoproteins and proteoglycans that form the **extracellular matrix (ECM)**, which functions in support, adhesion, movement, and regulation.
- Cell junctions connect neighboring cells. Plants have **plasmodesmata** that pass through adjoining cell walls. Animal cells have **tight junctions**, **desmosomes**, and **gap junctions**.

 Compare the structure and functions of a plant cell wall and the extracellular matrix of an animal cell.

CONCEPT 7.8

A cell is greater than the sum of its parts (pp. 191–193)

- Many components work together in a functioning cell.

 When a cell ingests a bacterium, what role does the nucleus play?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

6. **DRAW IT** From memory, draw two eukaryotic cells. Label the structures listed here and show any physical connections between the internal structures of each cell: nucleus, rough ER, smooth ER, mitochondrion, centrosome, chloroplast, vacuole, lysosome, microtubule, cell wall, ECM,

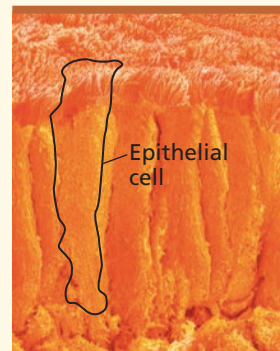


PRACTICE TEST
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microfilament, Golgi apparatus, intermediate filament, plasma membrane, peroxisome, ribosome, nucleolus, nuclear pore, vesicle, flagellum, microvilli, plasmodesma.

7. **EVOLUTION CONNECTION** (a) What cell structures best reveal evolutionary unity? (b) Provide an example of diversity related to specialized cellular modifications.
8. **SCIENTIFIC INQUIRY** Suppose you wish to separate two cellular proteins, A and B. Protein A binds with DNA and is found in the chromatin complex. Protein B binds with RNA and is found in nucleoli. How would you separate cell fractions containing protein A and protein B? Explain the rationale behind your method.
9. **WRITE ABOUT A THEME: ORGANIZATION** Considering some of the characteristics that define life and drawing on your knowledge of cellular structures and functions, write a short essay (100–150 words) that discusses this statement: Life is an emergent property that appears at the level of the cell. (See Concept 1.1.)

10. SYNTHESIZE YOUR KNOWLEDGE



The cells in this SEM are epithelial cells from the small intestine. Discuss how aspects of their structure contribute to their specialized functions of nutrient absorption and as a barrier between the intestinal contents and the blood supply on the other side of the sheet of epithelial cells.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Cell Membranes

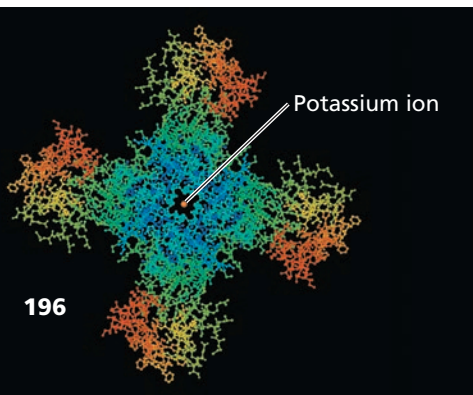
8

▲ **Figure 8.1** How do cell membrane proteins like this aquaporin (blue ribbons) help regulate chemical traffic?

KEY CONCEPTS

- 8.1** Cellular membranes are fluid mosaics of lipids and proteins
- 8.2** Membrane structure results in selective permeability
- 8.3** Passive transport is diffusion of a substance across a membrane with no energy investment
- 8.4** Active transport uses energy to move solutes against their gradients
- 8.5** Bulk transport across the plasma membrane occurs by exocytosis and endocytosis

▼ Potassium ion channel protein



Life at the Edge

The plasma membrane that surrounds the cell can be considered the edge of life, the boundary that separates a living cell from its surroundings and controls all inbound and outbound traffic. Like all biological membranes, the plasma membrane exhibits **selective permeability**; that is, it allows some substances to cross it more easily than others. The ability of the cell to discriminate in its chemical exchanges is fundamental to life, and it is the plasma membrane and its component molecules that make this selectivity possible.

In this chapter, you will learn how cellular membranes control the passage of substances, often with transport proteins. For example, the image in **Figure 8.1** shows a computer model of a short section of the phospholipid bilayer of a membrane (hydrophilic heads are yellow, and hydrophobic tails are green). The blue ribbons within the lipid bilayer represent helical regions of a membrane transport channel protein called an aquaporin. One molecule of this protein enables billions of water molecules (red and gray) to pass through the membrane every second, many more than could cross on their own. Another type of transport protein is the ion channel shown here; it allows potassium ions to pass through the membrane. To understand how the plasma membrane and its proteins enable cells to survive and function, we begin by examining membrane structure, then explore how plasma membranes control transport into and out of cells.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter



Animation: Membrane in Motion
Interview with Peter Agre: Discovering aquaporins

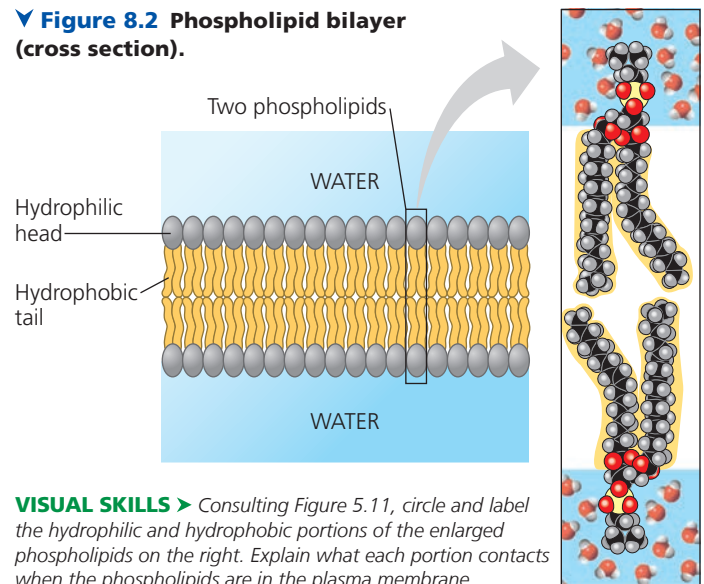
CONCEPT 8.1

Cellular membranes are fluid mosaics of lipids and proteins

Lipids and proteins are the staple ingredients of membranes, although carbohydrates are also important. The most abundant lipids in most membranes are phospholipids. The ability of phospholipids to form membranes is inherent in their molecular structure. A phospholipid is an **amphipathic** molecule, meaning it has both a hydrophilic (“water-loving”) region and a hydrophobic (“water-fearing”) region (see Figure 5.11). Other types of membrane lipids are also amphipathic. A phospholipid bilayer can exist as a stable boundary between two aqueous compartments because the molecular arrangement shelters the hydrophobic tails of the phospholipids from water while exposing the hydrophilic heads to water (**Figure 8.2**).

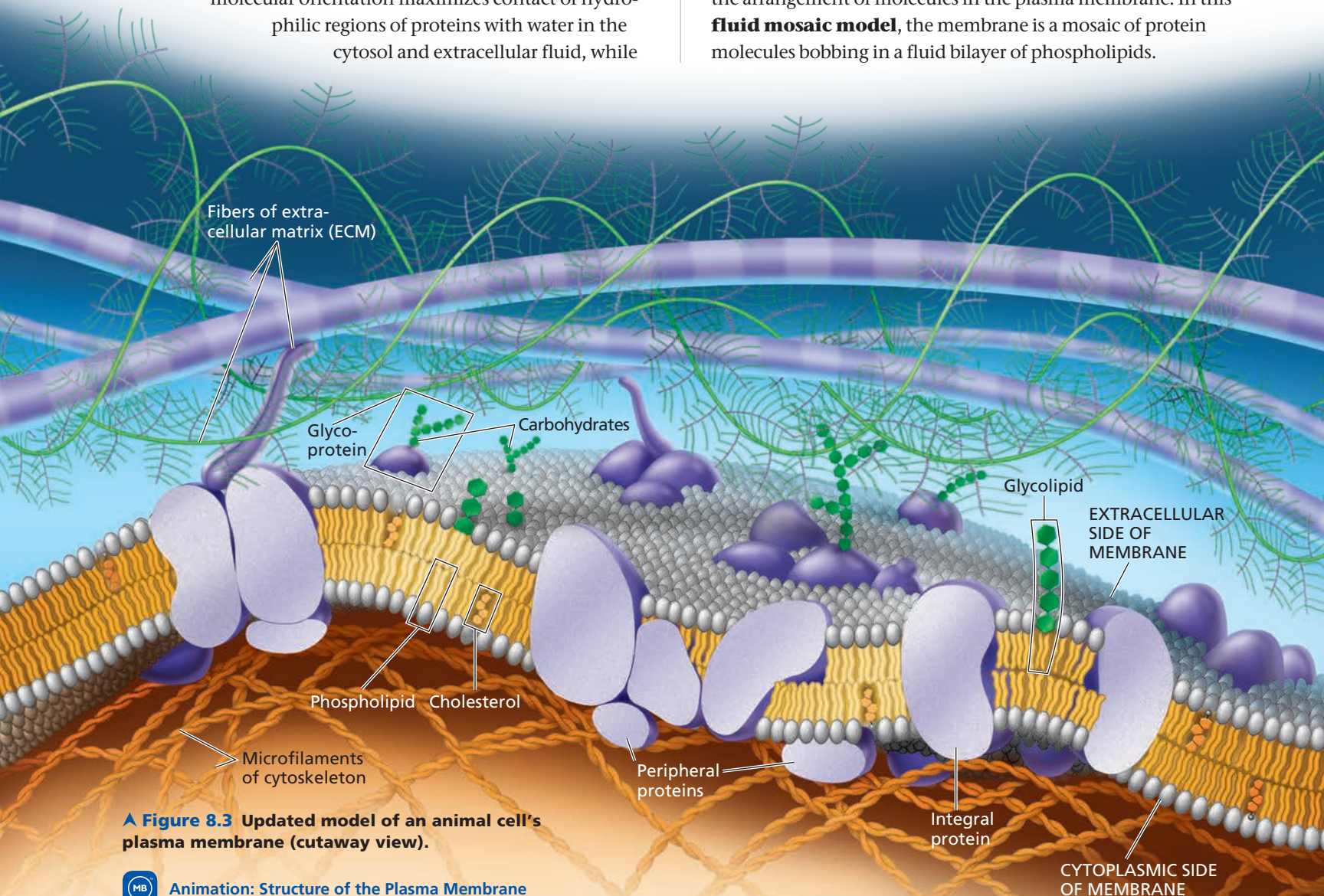
Like membrane lipids, most membrane proteins are amphipathic. Such proteins can reside in the phospholipid bilayer with their hydrophilic regions protruding. This molecular orientation maximizes contact of hydrophilic regions of proteins with water in the cytosol and extracellular fluid, while

▼ **Figure 8.2** Phospholipid bilayer (cross section).



VISUAL SKILLS ► Consulting Figure 5.11, circle and label the hydrophilic and hydrophobic portions of the enlarged phospholipids on the right. Explain what each portion contacts when the phospholipids are in the plasma membrane.

providing their hydrophobic parts with a nonaqueous environment. **Figure 8.3** shows the currently accepted model of the arrangement of molecules in the plasma membrane. In this **fluid mosaic model**, the membrane is a mosaic of protein molecules bobbing in a fluid bilayer of phospholipids.



▲ **Figure 8.3** Updated model of an animal cell's plasma membrane (cutaway view).



The proteins are not randomly distributed in the membrane, however. Groups of proteins are often associated in long-lasting, specialized patches, where they carry out common functions. Researchers have found specific lipids in these patches as well and have proposed naming them *lipid rafts*, but there is ongoing controversy about whether such structures exist in living cells or are an artifact of biochemical techniques. Like all models, the fluid mosaic model is continually being refined as new research reveals more about membrane structure.

The Fluidity of Membranes

Membranes are not static sheets of molecules locked rigidly in place. A membrane is held together mainly by hydrophobic interactions, which are much weaker than covalent bonds (see Figure 5.18). Most of the lipids and some proteins can shift about sideways—that is, in the plane of the membrane, like partygoers elbowing their way through a crowded room. Very rarely, also, a lipid may flip-flop across the membrane, switching from one phospholipid layer to the other.

The sideways movement of phospholipids within the membrane is rapid. Adjacent phospholipids switch positions about 10^7 times per second, which means that a phospholipid can travel about $2\ \mu\text{m}$ —the length of many bacterial cells—in 1 second. Proteins are much larger than lipids and move more slowly, but some membrane proteins do drift, as shown in a classic experiment described in Figure 8.4. Some membrane proteins seem to move in a highly directed manner, perhaps driven along cytoskeletal fibers in the cell by motor proteins

connected to the membrane proteins' cytoplasmic regions. However, many other membrane proteins seem to be held immobile by their attachment to the cytoskeleton or to the extracellular matrix (see Figure 8.3).

A membrane remains fluid as temperature decreases until the phospholipids settle into a closely packed arrangement and the membrane solidifies, much as bacon grease forms lard when it cools. The temperature at which a membrane solidifies depends on the types of lipids it is made of. As the temperature decreases, the membrane remains fluid to a lower temperature if it is rich in phospholipids with unsaturated hydrocarbon tails (see Figures 5.10 and 5.11). Because of kinks in the tails where double bonds are located, unsaturated hydrocarbon tails cannot pack together as closely as saturated hydrocarbon tails, making the membrane more fluid (Figure 8.5a).

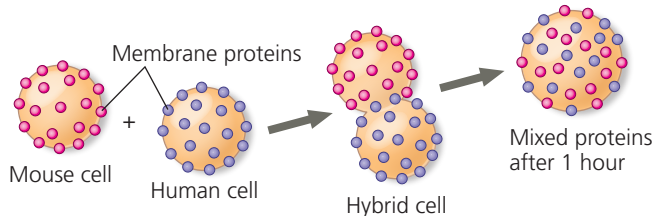
The steroid cholesterol, which is wedged between phospholipid molecules in the plasma membranes of animal cells, has different effects on membrane fluidity at different temperatures (Figure 8.5b). At relatively high temperatures—at 37°C , the body temperature of humans, for example—cholesterol makes the membrane less fluid by restraining phospholipid movement. However, because cholesterol also hinders the close packing of phospholipids, it lowers the temperature required for the membrane to solidify. Thus, cholesterol can be thought of as a “fluidity buffer” for the membrane, resisting changes in membrane fluidity that can be caused by changes in temperature. Compared to animals, plants have very low levels of cholesterol; rather, related steroid lipids buffer membrane fluidity in plant cells.

▼ Figure 8.4

Inquiry Do membrane proteins move?

Experiment Larry Frye and Michael Edidin, at Johns Hopkins University, labeled the plasma membrane proteins of a mouse cell and a human cell with two different markers and fused the cells. Using a microscope, they observed the markers on the hybrid cell.

Results



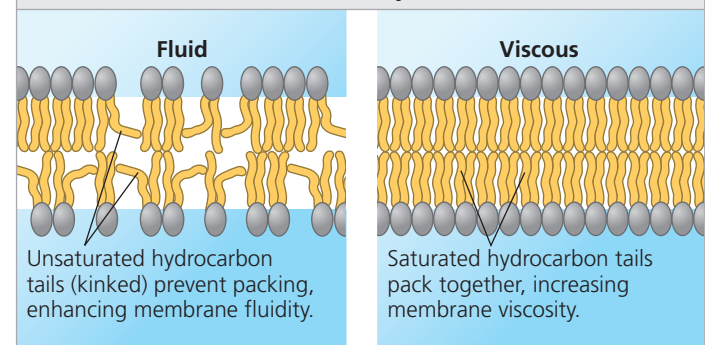
Conclusion The mixing of the mouse and human membrane proteins indicates that at least some membrane proteins move sideways within the plane of the plasma membrane.

Data from L. D. Frye and M. Edidin, The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons, *Journal of Cell Science* 7:319 (1970).

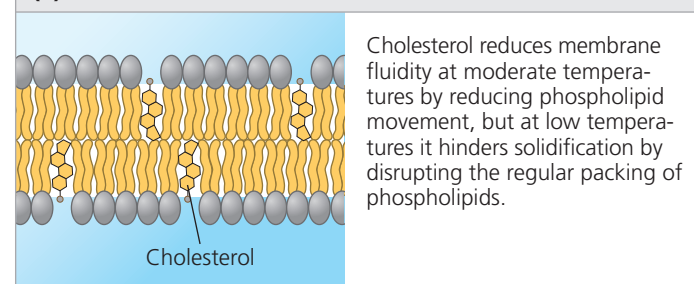
WHAT IF? > Suppose the proteins did not mix in the hybrid cell, even many hours after fusion. Would you be able to conclude that proteins don't move within the membrane? What other explanation could there be?

▼ Figure 8.5 Factors that affect membrane fluidity.

(a) Unsaturated versus saturated hydrocarbon tails.



(b) Cholesterol within the animal cell membrane.



Membranes must be fluid to work properly; the fluidity of a membrane affects both its permeability and the ability of membrane proteins to move to where their function is needed. Usually, membranes are about as fluid as salad oil. When a membrane solidifies, its permeability changes, and enzymatic proteins in the membrane may become inactive if their activity requires movement within the membrane. However, membranes that are too fluid cannot support protein function either. Therefore, extreme environments pose a challenge for life, resulting in evolutionary adaptations that include differences in membrane lipid composition.

Evolution of Differences in Membrane Lipid Composition

EVOLUTION Variations in the cell membrane lipid compositions of many species appear to be evolutionary adaptations that maintain the appropriate membrane fluidity under specific environmental conditions. For instance, fishes that live in extreme cold have membranes with a high proportion of unsaturated hydrocarbon tails, enabling their membranes to remain fluid (see Figure 8.5a). At the other extreme, some bacteria and archaea thrive at temperatures greater than 90°C (194°F) in thermal hot springs and geysers. Their membranes include unusual lipids that may prevent excessive fluidity at such high temperatures.

The ability to change the lipid composition of cell membranes in response to changing temperatures has evolved in organisms that live where temperatures vary. In many plants that tolerate extreme cold, such as winter wheat, the percentage of unsaturated phospholipids increases in autumn, an adjustment that keeps the membranes from solidifying during winter. Certain bacteria and archaea can also change the proportion of unsaturated phospholipids in their cell membranes, depending on the temperature at which they are growing. Overall, natural selection has apparently favored organisms whose mix of membrane lipids ensures an appropriate level of membrane fluidity for their environment.

Membrane Proteins and Their Functions

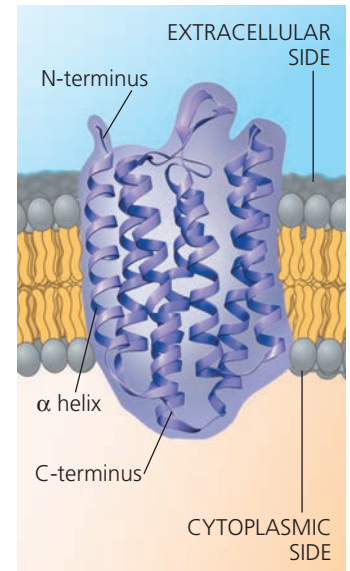
Now we come to the *mosaic* aspect of the fluid mosaic model. Somewhat like a tile mosaic (shown here), a membrane is a collage of different proteins, often clustered together in groups, embedded in the fluid matrix of the lipid bilayer (see Figure 8.3). In the plasma

membrane of red blood cells alone,

for example, more than 50 kinds of proteins have been found so far. Phospholipids form the main fabric of the membrane, but proteins determine most of the membrane's functions. Different types of cells contain different sets of membrane proteins, and the

► Figure 8.6 The structure of a transmembrane protein.

Bacteriorhodopsin (a bacterial transport protein) has a distinct orientation in the membrane, with its N-terminus outside the cell and its C-terminus inside. This ribbon model highlights the secondary structure of the hydrophobic parts, including seven transmembrane α helices, which lie mostly within the hydrophobic interior of the membrane. The nonhelical hydrophilic segments are in contact with the aqueous solutions on the extracellular and cytoplasmic sides of the membrane.

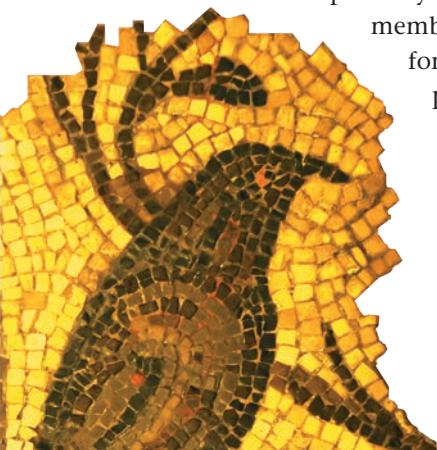


various membranes within a cell each have a unique collection of proteins.

Notice in Figure 8.3 that there are two major populations of membrane proteins: integral proteins and peripheral proteins. **Integral proteins** penetrate the hydrophobic interior of the lipid bilayer. The majority are *transmembrane proteins*, which span the membrane; other integral proteins extend only part-way into the hydrophobic interior. The hydrophobic regions of an integral protein consist of one or more stretches of non-polar amino acids (see Figure 5.14), typically 20–30 amino acids in length, usually coiled into α helices (Figure 8.6). The hydrophilic parts of the molecule are exposed to the aqueous solutions on either side of the membrane. Some proteins also have one or more hydrophilic channels that allow passage through the membrane of hydrophilic substances (even of water itself; see Figure 8.1). **Peripheral proteins** are not embedded in the lipid bilayer at all; they are loosely bound to the surface of the membrane, often to exposed parts of integral proteins (see Figure 8.3).

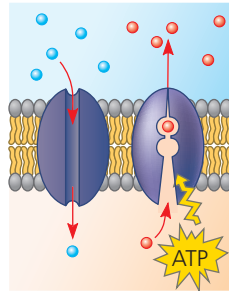
On the cytoplasmic side of the plasma membrane, some membrane proteins are held in place by attachment to the cytoskeleton. And on the extracellular side, certain membrane proteins may attach to materials outside the cell. For example, in animal cells, membrane proteins may be attached to fibers of the extracellular matrix (see Figure 7.28; *integrins* are one type of integral, transmembrane protein). These attachments combine to give animal cells a stronger framework than the plasma membrane alone could provide.

A single cell may have cell-surface membrane proteins that carry out several different functions, such as transport through the cell membrane, enzymatic activity, or attaching a cell to either a neighboring cell or the extracellular matrix. Furthermore, a single membrane protein may itself carry out multiple functions. Thus, the membrane is not only a structural mosaic, with many proteins embedded in the membrane, but also a functional mosaic, carrying out a range

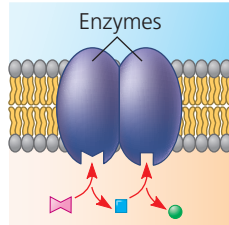


▼ **Figure 8.7 Some functions of membrane proteins.** In many cases, a single protein performs multiple tasks.

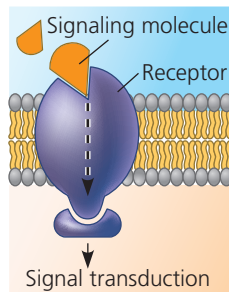
(a) Transport. *Left:* A protein that spans the membrane may provide a hydrophilic channel across the membrane that is selective for a particular solute. *Right:* Other transport proteins shuttle a substance from one side to the other by changing shape (see Figure 8.14b). Some of these proteins hydrolyze ATP as an energy source to actively pump substances across the membrane.



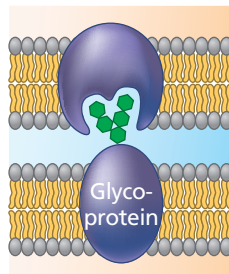
(b) Enzymatic activity. A protein built into the membrane may be an enzyme with its active site (where the reactant binds) exposed to substances in the adjacent solution. In some cases, several enzymes in a membrane are organized as a team that carries out sequential steps of a metabolic pathway.



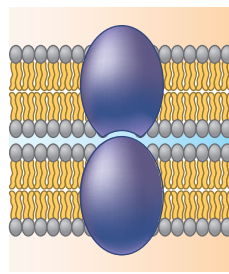
(c) Signal transduction. A membrane protein (receptor) may have a binding site with a specific shape that fits the shape of a chemical messenger, such as a hormone. The external messenger (signaling molecule) may cause the protein to change shape, allowing it to relay the message to the inside of the cell, usually by binding to a cytoplasmic protein (see Figure 9.6).



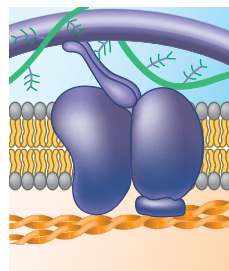
(d) Cell-cell recognition. Some glycoproteins serve as identification tags that are specifically recognized by membrane proteins of other cells. This type of cell-cell binding is usually short-lived compared to that shown in (e).



(e) Intercellular joining. Membrane proteins of adjacent cells may hook together in various kinds of junctions, such as gap junctions or tight junctions (see Figure 7.30). This type of binding is more long-lasting than that shown in (d).



(f) Attachment to the cytoskeleton and extracellular matrix (ECM). Microfilaments or other elements of the cytoskeleton may be noncovalently bound to membrane proteins, a function that helps maintain cell shape and stabilizes the location of certain membrane proteins. Proteins that can bind to ECM molecules can coordinate extracellular and intracellular changes (see Figure 7.28).



VISUAL SKILLS ► Some transmembrane proteins can bind to a particular ECM molecule and, when bound, transmit a signal into the cell. Use the proteins shown in (c) and (f) to explain how this might occur.

of functions. **Figure 8.7** illustrates six major functions performed by proteins of the plasma membrane.

Animation: Functions of the Plasma Membrane

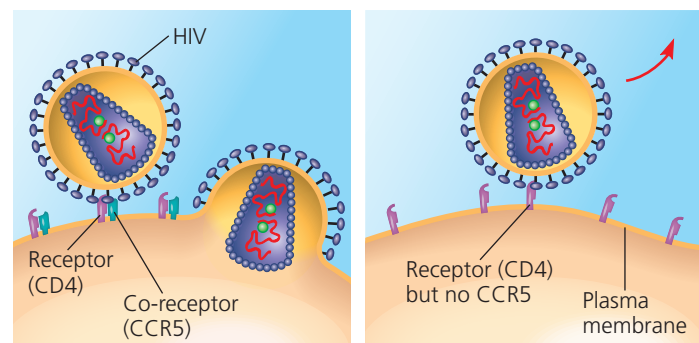
Proteins on a cell's surface are important in the medical field. For example, a protein called CD4 on the surface of immune cells helps the human immunodeficiency virus (HIV) infect these cells, leading to acquired immune deficiency syndrome (AIDS). Despite multiple exposures to HIV, however, a small number of people do not develop AIDS and show no evidence of HIV-infected cells. Comparing their genes with the genes of infected individuals, researchers learned that resistant people have an unusual form of a gene that codes for an immune cell-surface protein called CCR5. Further work showed that although CD4 is the main HIV receptor, HIV must also bind to CCR5 as a “co-receptor” to infect most cells (**Figure 8.8a**). An absence of CCR5 on the cells of resistant individuals, due to the gene alteration, prevents the virus from entering the cells (**Figure 8.8b**).

This information has been key to developing a treatment for HIV infection. Interfering with CD4 causes dangerous side effects because of its many important functions in cells. Discovery of the CCR5 co-receptor provided a safer target for development of drugs that mask this protein and block HIV entry. One such drug, maraviroc (brand name Selzentry), was approved for treatment of HIV in 2007 and is now being tested to determine whether this drug might also work to prevent HIV infection in uninfected, at-risk patients.

The Role of Membrane Carbohydrates in Cell-Cell Recognition

Cell-cell recognition, a cell's ability to distinguish one type of neighboring cell from another, is crucial to the functioning of an organism. It is important, for example, in the sorting of cells into tissues and organs in an animal embryo. It is also

▼ **Figure 8.8 The genetic basis for HIV resistance.**



(a) HIV can infect a cell with CCR5 on its surface, as in most people.

(b) HIV cannot infect a cell lacking CCR5 on its surface, as in resistant individuals.

MAKE CONNECTIONS ► Study Figures 2.16 and 5.17; each shows pairs of molecules binding to each other. What would you predict about CCR5 that would allow HIV to bind to it? How could a drug molecule interfere with this binding?

the basis for the rejection of foreign cells by the immune system, an important line of defense in vertebrate animals (see Concept 47.1). Cells recognize other cells by binding to molecules, often containing carbohydrates, on the extracellular surface of the plasma membrane (see Figure 8.7d).

Membrane carbohydrates are usually short, branched chains of fewer than 15 sugar units. Some are covalently bonded to lipids, forming molecules called **glycolipids**. (Recall that *glyco* refers to carbohydrate.) However, most are covalently bonded to proteins, which are thereby **glycoproteins** (see Figure 8.3).

The carbohydrates on the extracellular side of the plasma membrane vary from species to species, among individuals of the same species, and even from one cell type to another in a single individual. The diversity of the molecules and their location on the cell's surface enable membrane carbohydrates to function as markers that distinguish one cell from another. For example, the four human blood types designated A, B, AB, and O reflect variation in the carbohydrate part of glycoproteins on the surface of red blood cells.

Synthesis and Sidedness of Membranes

Membranes have distinct inside and outside faces. The two lipid layers may differ in lipid composition, and each protein has directional orientation in the membrane (see Figure 8.6).

Figure 8.9 shows how membrane sidedness arises: The asymmetrical arrangement of proteins, lipids, and their

associated carbohydrates in the plasma membrane is determined as the membrane is being built by the endoplasmic reticulum (ER) and Golgi apparatus, components of the endomembrane system (see Figure 7.15).

CONCEPT CHECK 8.1

- 1. VISUAL SKILLS** > Carbohydrates are attached to plasma membrane proteins in the ER (see Figure 8.9). On which side of the vesicle membrane are the carbohydrates during transport to the cell surface?
- 2. WHAT IF?** > How would the membrane lipid composition of a native grass found in very warm soil around hot springs compare with that of a native grass found in cooler soil? Explain.

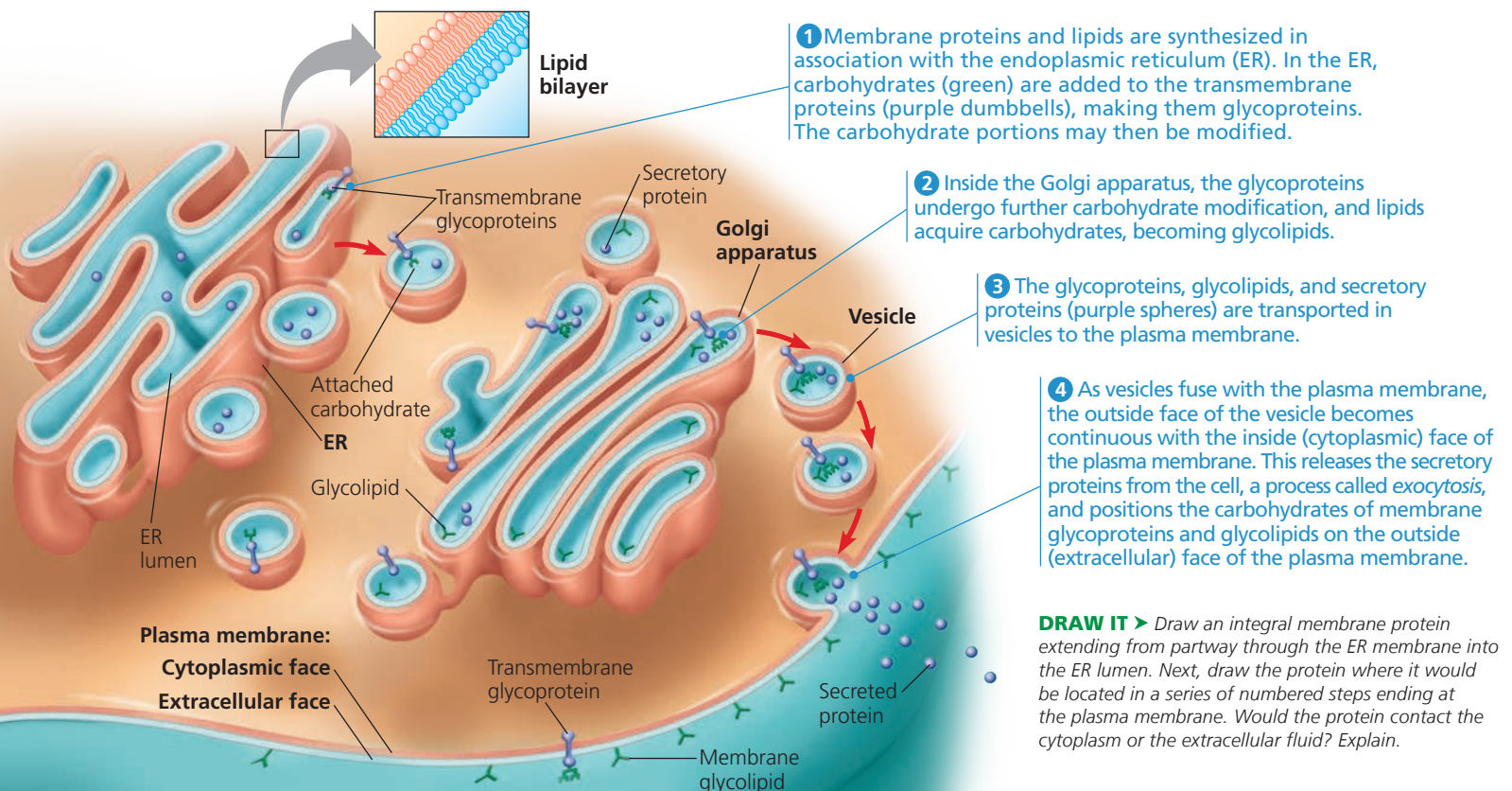
For suggested answers, see Appendix A.

CONCEPT 8.2

Membrane structure results in selective permeability

The biological membrane is an exquisite example of a supramolecular structure—many molecules ordered into a higher level of organization—with emergent properties beyond those of the individual molecules. The remainder of this chapter focuses on one of those properties: the ability to regulate transport across cellular boundaries, a function essential to the cell's existence. We will see once again that form fits

▼ **Figure 8.9 Synthesis of membrane components and their orientation in the membrane.** The cytoplasmic (orange) face of the plasma membrane differs from the extracellular (aqua) face. The latter arises from the inside face of ER, Golgi, and vesicle membranes.



function: The fluid mosaic model helps explain how membranes regulate the cell's molecular traffic.

A steady traffic of small molecules and ions moves across the plasma membrane in both directions. Consider the chemical exchanges between a muscle cell and the extracellular fluid that bathes it. Sugars, amino acids, and other nutrients enter the cell, and metabolic waste products leave it. The cell takes in O_2 for use in cellular respiration and expels CO_2 . Also, the cell regulates its concentrations of inorganic ions, such as Na^+ , K^+ , Ca^{2+} , and Cl^- , by shuttling them one way or the other across the plasma membrane. Although the heavy traffic through them may seem to suggest otherwise, cell membranes are selectively permeable, and substances do not cross the barrier indiscriminately. The cell is able to take up some small molecules and ions and exclude others.

The Permeability of the Lipid Bilayer

Nonpolar molecules, such as hydrocarbons, CO_2 , and O_2 , are hydrophobic, as are lipids. They can all therefore dissolve in the lipid bilayer of the membrane and cross it easily, without the aid of membrane proteins. However, the hydrophobic interior of the membrane impedes direct passage through the membrane of ions and polar molecules, which are hydrophilic. Polar molecules such as glucose and other sugars pass only slowly through a lipid bilayer, and even water, a very small polar molecule, does not cross rapidly relative to nonpolar molecules. A charged atom or molecule and its surrounding shell of water (see Figure 3.8) are even less likely to penetrate the hydrophobic interior of the membrane. Furthermore, the lipid bilayer is only one aspect of the gatekeeper system responsible for a cell's selective permeability. Proteins built into the membrane play key roles in regulating transport.

Transport Proteins

Specific ions and a variety of polar molecules can't move through cell membranes on their own. However, these hydrophilic substances can avoid contact with the lipid bilayer by passing through **transport proteins** that span the membrane.

Some transport proteins, called *channel proteins*, function by having a hydrophilic channel that certain molecules or atomic ions use as a tunnel through the membrane (see Figure 8.7a, left). For example, the passage of water molecules through the membrane in certain cells is greatly facilitated by channel proteins known as **aquaporins** (see Figure 8.1). Each aquaporin allows entry of up to 3 *billion* (3×10^9) water molecules per second, passing single file through its central channel, which fits ten at a time. Without aquaporins, only a tiny fraction of these water molecules would pass through the same area of the cell membrane in a second, so the channel protein brings about a tremendous increase in rate. Other transport proteins, called *carrier proteins*, hold onto their passengers and change shape in a way that shuttles them across the membrane (see Figure 8.7a, right).

A transport protein is specific for the substance it translocates (moves), allowing only a certain substance (or a small group of related substances) to cross the membrane. For example, a specific carrier protein in the plasma membrane of red blood cells transports glucose across the membrane 50,000 times faster than glucose can pass through on its own. This "glucose transporter" is so selective that it even rejects fructose, a structural isomer of glucose. Thus, the selective permeability of a membrane depends on both the discriminating barrier of the lipid bilayer and the specific transport proteins built into the membrane.



Animation: Selective Permeability of Membranes

What establishes the *direction* of traffic across a membrane? And what mechanisms drive molecules across membranes? We will address these questions next as we explore two modes of membrane traffic: passive transport and active transport.

CONCEPT CHECK 8.2

1. What property allows O_2 and CO_2 to cross a lipid bilayer without the aid of membrane proteins?
2. **VISUAL SKILLS** > Examine Figure 8.2. Why is a transport protein needed to move many water molecules rapidly across a membrane?
3. **MAKE CONNECTIONS** > Aquaporins exclude passage of hydronium ions (H_3O^+), but some aquaporins allow passage of glycerol, a three-carbon alcohol (see Figure 5.9), as well as H_2O . Since H_3O^+ is closer in size to water than glycerol is, yet cannot pass through, what might be the basis of this selectivity?

For suggested answers, see Appendix A.

CONCEPT 8.3

Passive transport is diffusion of a substance across a membrane with no energy investment

Molecules have a type of energy called thermal energy, due to their constant motion (see Concept 3.2). One result of this motion is **diffusion**, the movement of particles of any substance so that they spread out into the available space. Each molecule moves randomly, yet diffusion of a *population* of molecules may be directional. To understand this process, let's imagine a synthetic membrane separating pure water from a solution of a dye in water. Study **Figure 8.10a** carefully to appreciate how diffusion would result in both solutions having equal concentrations of the dye molecules. Once that point is reached, there will be a dynamic equilibrium, with roughly as many dye molecules crossing the membrane each second in one direction as in the other.

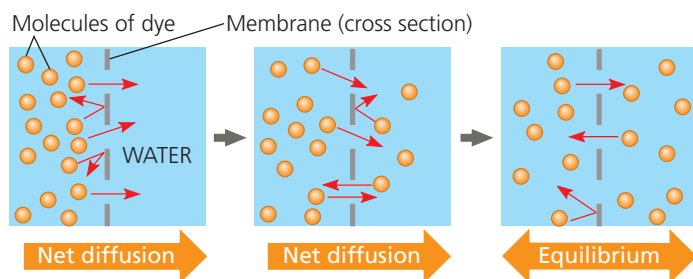
We can now state a simple rule of diffusion: In the absence of other forces, a substance will diffuse from where it is more concentrated to where it is less concentrated. Put another way, any substance will diffuse down its **concentration gradient**,

the region along which the density of a chemical substance increases or decreases (in this case, decreases). No work must be done to make this happen; diffusion is a spontaneous process, needing no input of energy. Note that each substance diffuses down its *own* concentration gradient, unaffected by the concentration gradients of other substances (**Figure 8.10b**).

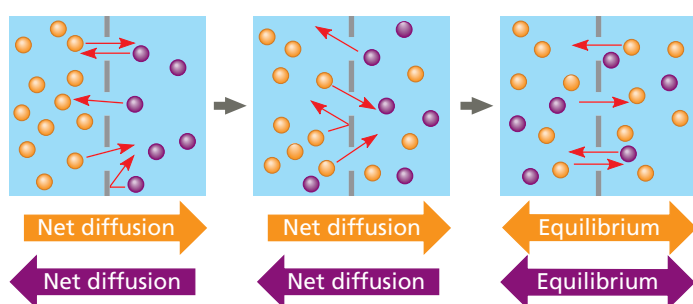
Much of the traffic across cell membranes occurs by diffusion. When a substance is more concentrated on one side of a membrane than on the other, there is a tendency for the substance to diffuse across the membrane down its concentration gradient (assuming that the membrane is permeable to that substance). One important example is the uptake of oxygen by a cell performing cellular respiration. Dissolved oxygen diffuses into the cell across the plasma membrane. As long as cellular respiration consumes the O_2 as it enters, diffusion into the cell will continue because the concentration gradient favors movement in that direction.

The diffusion of a substance across a biological membrane is called **passive transport** because the cell does not have

▼ **Figure 8.10 The diffusion of solutes across a synthetic membrane.** Each of the large arrows under the diagrams shows the net diffusion of the dye molecules of that color.



(a) **Diffusion of one solute.** The membrane has pores large enough for molecules of dye to pass through. Random movement of dye molecules will cause some to pass through the pores; this will happen more often on the side with more dye molecules. The dye diffuses from where it is more concentrated to where it is less concentrated (called diffusing down a concentration gradient). This leads to a dynamic equilibrium: The solute molecules continue to cross the membrane, but at roughly equal rates in both directions.



(b) **Diffusion of two solutes.** Solutions of two different dyes are separated by a membrane that is permeable to both. Each dye diffuses down its own concentration gradient. There will be a net diffusion of the purple dye toward the left, even though the *total* solute concentration was initially greater on the left side.

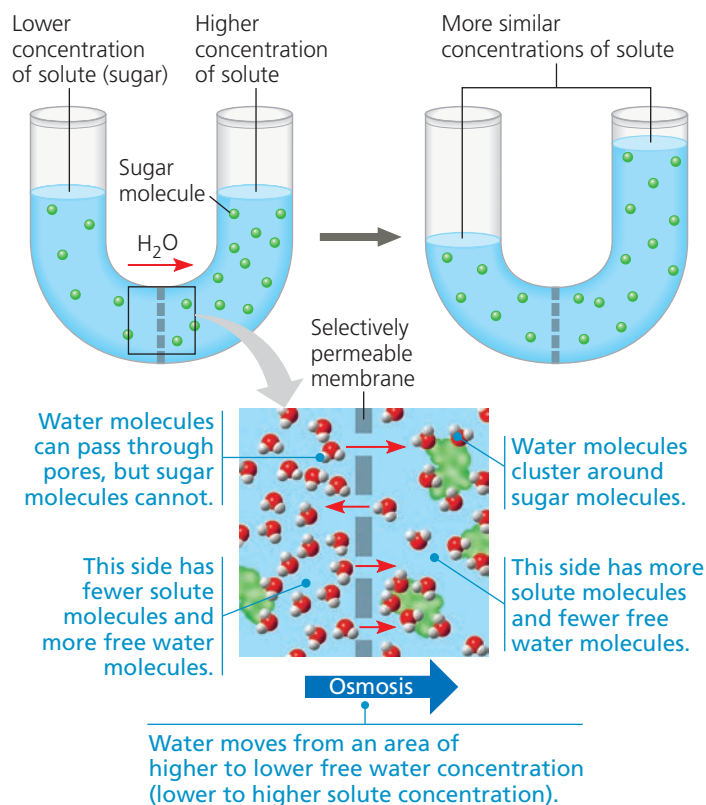
Animation: Diffusion

to expend energy to make it happen. The concentration gradient itself represents potential energy (see Concept 2.2 and Figure 6.5b) and drives diffusion. Remember, however, that membranes are selectively permeable and therefore have different effects on the rates of diffusion of various molecules. In the case of water, the presence of aquaporin proteins allows water to diffuse very rapidly across the membranes of certain cells compared with diffusion in the absence of aquaporins. As we'll see next, the movement of water across the plasma membrane has important consequences for cells.

Effects of Osmosis on Water Balance

To see how two solutions with different solute concentrations interact, picture a U-shaped glass tube with a selectively permeable artificial membrane separating two sugar solutions (**Figure 8.11**). Pores in this synthetic membrane are too small

▼ **Figure 8.11 Osmosis.** Two sugar solutions of different concentrations are separated by a membrane that the solvent (water) can pass through but the solute (sugar) cannot. Water molecules move randomly and may cross in either direction, but overall, water diffuses from the solution with less concentrated solute to that with more concentrated solute. This passive transport of water, or osmosis, makes the sugar concentrations on both sides more nearly equal. (The concentrations are prevented from being exactly equal due to the effect of water pressure on the higher side, which is not discussed here, for simplicity.)



VISUAL SKILLS ► If an orange dye capable of passing through the membrane was added to the left side of the tube above, how would it be distributed at the end of the experiment? (See Figure 8.10.) Would the final solution levels in the tube be affected?

Figure Walkthrough

for sugar molecules to pass through but large enough for water molecules. However, tight clustering of water molecules around the hydrophilic solute molecules makes some of the water unavailable to cross the membrane. As a result, the solution with a higher solute concentration has a lower *free* water concentration. Water diffuses across the membrane from the region of higher free water concentration (lower solute concentration) to that of lower free water concentration (higher solute concentration) until the solute concentrations on both sides of the membrane are more nearly equal. The diffusion of free water across a selectively permeable membrane, whether artificial or cellular, is called **osmosis**. The movement of water across cell membranes and the balance of water between the cell and its environment are crucial to organisms. Let's now apply what we've learned about osmosis in this system to living cells.

Water Balance of Cells Without Cell Walls

To explain the behavior of a cell in a solution, we must consider both solute concentration and membrane permeability. Both factors are taken into account in the concept of **tonicity**, the ability of a surrounding solution to cause a cell to gain or lose water. The tonicity of a solution depends in part on its concentration of solutes that cannot cross the membrane (nonpenetrating solutes) relative to that inside the cell. If there is a higher concentration of nonpenetrating solutes in the surrounding solution, water will tend to leave the cell, and vice versa.

If a cell without a cell wall, such as an animal cell, is immersed in an environment that is **isotonic** to the cell (*iso* means “same”), there will be no *net* movement of water across the plasma membrane. Water diffuses across the membrane, but at the same rate in both directions. In an isotonic environment, the volume of an animal cell is stable (**Figure 8.12a**).

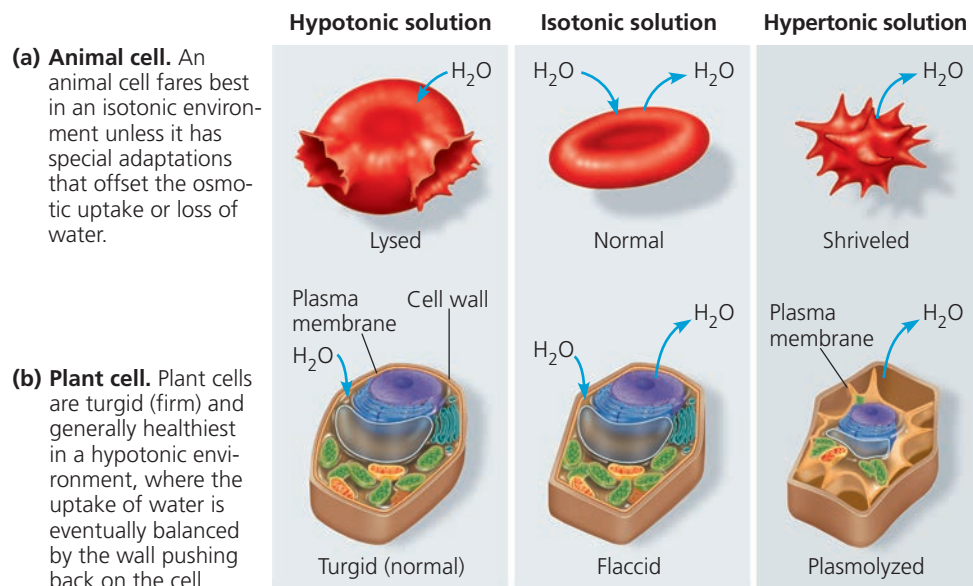
Let's transfer the cell to a solution that is **hypertonic** to the cell (*hyper* means “more,” in this case referring to nonpenetrating solutes). The cell will lose water, shrivel, and probably die. This is why an increase in the salinity (saltiness) of a lake can kill the animals there; if the lake water becomes hypertonic to the animals' cells, they might shrivel and die. However, taking up too much water can be just as hazardous as losing water. If we place the cell in a solution that is **hypotonic** to the cell (*hypo* means “less”), water will enter the cell faster than it leaves, and the cell will swell and lyse (burst) like an overfilled water balloon.

A cell without rigid cell walls can tolerate neither excessive uptake nor excessive loss of water. This problem of water balance is automatically solved if such a cell lives in isotonic surroundings. Seawater is isotonic to many marine invertebrates. The cells of most terrestrial (land-dwelling) animals are bathed in an extracellular fluid that is isotonic to the cells. In hypertonic or hypotonic environments, however, organisms that lack rigid cell walls must have other adaptations for **osmoregulation**, the control of solute concentrations and water balance. For example, the unicellular eukaryote *Paramecium* lives in pond water, which is hypotonic to the cell. *Paramecium* has a plasma membrane that is much less permeable to water than the membranes of most other cells, but this only slows the uptake of water, which continually enters the cell. The *Paramecium* cell doesn't burst because it is also equipped with a contractile vacuole, an organelle that functions as a bilge pump to force water out of the cell as fast as it enters by osmosis (**Figure 8.13**). In contrast, the bacteria and archaea that live in hypersaline (excessively salty) environments (see Figure 27.1) have cellular mechanisms that balance the internal and external solute concentrations to ensure that water does not move out of the cell. We will examine other evolutionary adaptations for osmoregulation by animals in Concept 44.1.

Water Balance of Cells with Cell Walls

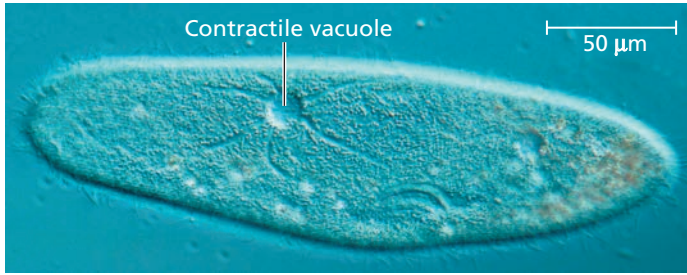
The cells of plants, prokaryotes, fungi, and some unicellular eukaryotes are surrounded by cell walls (see Figure 7.27). When such a cell is immersed in a hypotonic solution—bathed in rainwater, for example—the cell wall helps maintain the cell's

▼ **Figure 8.12 The water balance of living cells.** How living cells react to changes in the solute concentration of their environment depends on whether or not they have cell walls. (a) Animal cells, such as this red blood cell, do not have cell walls. (b) Plant cells do have cell walls. (Arrows indicate net water movement after the cells were first placed in these solutions.)



Animation: Osmosis and Water Balance in Cells
Video: Turgid Elodea
Video: Plasmolysis in Elodea

▼ **Figure 8.13 The contractile vacuole of *Paramecium*.** The vacuole collects fluid from canals in the cytoplasm. When full, the vacuole and canals contract, expelling fluid from the cell (LM).



 **Video: *Paramecium* Vacuole**

water balance. Consider a plant cell. Like an animal cell, the plant cell swells as water enters by osmosis (**Figure 8.12b**). However, the relatively inelastic cell wall will expand only so much before it exerts a back pressure on the cell, called *turgor pressure*, that opposes further water uptake. At this point, the cell is **turgid** (very firm), the healthy state for most plant cells. Plants that are not woody, such as most houseplants, depend for mechanical support on cells kept turgid by a surrounding hypotonic solution. If a plant's cells and surroundings are isotonic, there is no net tendency for water to enter and the cells become **flaccid** (limp); the plant wilts.

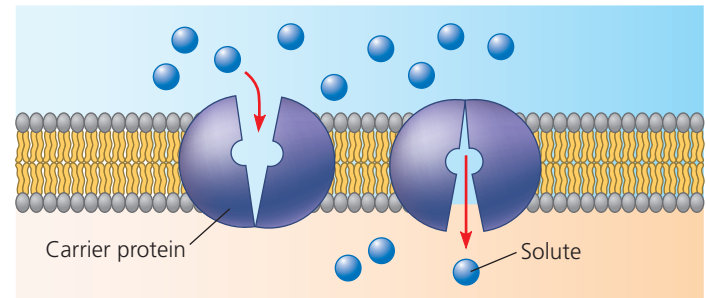
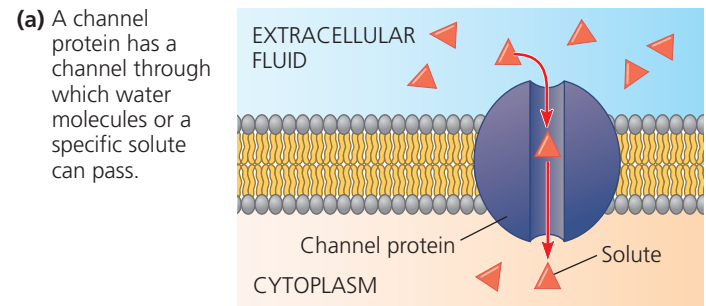
However, a cell wall is of no advantage if the cell is immersed in a hypertonic environment. In this case, a plant cell, like an animal cell, will lose water to its surroundings and shrink. As the plant cell shrivels, its plasma membrane pulls away from the cell wall at multiple places. This phenomenon, called **plasmolysis**, causes the plant to wilt and can lead to plant death. The walled cells of bacteria and fungi also plasmolyze in hypertonic environments.

Facilitated Diffusion: Passive Transport Aided by Proteins

Let's look more closely at how water and certain hydrophilic solutes cross a membrane. As mentioned earlier, many polar molecules and ions impeded by the lipid bilayer of the membrane diffuse passively with the help of transport proteins that span the membrane. This phenomenon is called **facilitated diffusion**. Cell biologists are still trying to learn exactly how various transport proteins facilitate diffusion. Most transport proteins are very specific: They transport some substances but not others.

As mentioned earlier, the two types of transport proteins are channel proteins and carrier proteins. Channel proteins simply provide corridors that allow specific molecules or ions to cross the membrane (**Figure 8.14a**). The hydrophilic passageways provided by these proteins can allow water molecules or small ions to diffuse very quickly from one side of the membrane to the other. Aquaporins, the water channel proteins, facilitate the massive levels of diffusion of water (osmosis) that occur in plant cells and in animal cells such as

▼ **Figure 8.14 Two types of transport proteins that carry out facilitated diffusion.** In both cases, the protein can transport the solute in either direction, but the net movement is down the concentration gradient of the solute.



(b) A carrier protein alternates between two shapes, moving a solute across the membrane during the shape change.

 **Animation: Facilitated Diffusion**

red blood cells (see Figure 8.12). Certain kidney cells also have a high number of aquaporins, allowing them to reclaim water from urine before it is excreted. If the kidneys did not perform this function, you would excrete about 180 L of urine per day—and have to drink an equal volume of water!

Channel proteins that transport ions are called **ion channels**. Many ion channels function as **gated channels**, which open or close in response to a stimulus. For some gated channels, the stimulus is electrical. In a nerve cell, for example, an ion channel opens in response to an electrical stimulus, allowing a stream of potassium ions to leave the cell. (See the potassium ion channel at the beginning of this chapter.) This restores the cell's ability to fire again. Other gated channels open or close when a specific substance other than the one to be transported binds to the channel. These gated channels are also important in the functioning of the nervous system, as you'll learn in Concepts 48.2 and 48.3.

 **Interview with Elba Serrano: Investigating how ion channels enable you to hear**

Carrier proteins, such as the glucose transporter mentioned earlier, seem to undergo a subtle change in shape that somehow translocates the solute-binding site across the membrane (**Figure 8.14b**). Such a change in shape may be triggered by the binding and release of the transported molecule. Like ion channels, carrier proteins involved in facilitated diffusion result in the net movement of a substance down its concentration gradient.

SCIENTIFIC SKILLS EXERCISE

Interpreting a Scatter Plot with Two Sets of Data

Is Glucose Uptake into Cells Affected by Age? Glucose, an important energy source for animals, is transported into cells by facilitated diffusion using protein carriers. In this exercise, you will interpret a graph with two sets of data from an experiment that examined glucose uptake over time in red blood cells from guinea pigs of different ages. You will determine if the cells' rate of glucose uptake depended on the age of the guinea pigs.

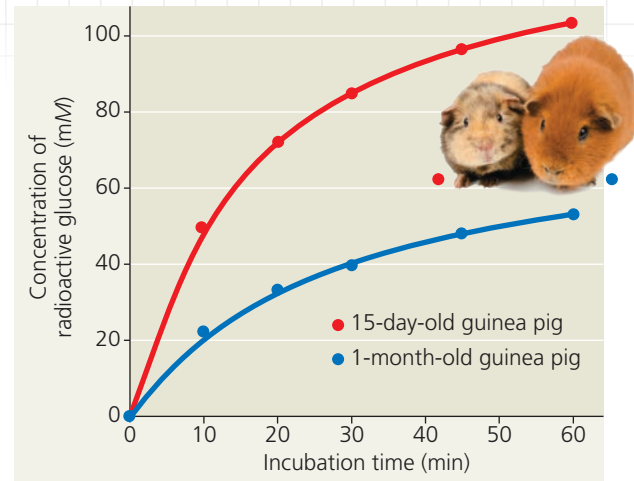
How the Experiment Was Done Researchers incubated guinea pig red blood cells in a 300 mM (millimolar) radioactive glucose solution at pH 7.4 at 25°C. Every 10 or 15 minutes, they removed a sample of cells and measured the concentration of radioactive glucose inside those cells. The cells came from either a 15-day-old or a 1-month-old guinea pig.

Data from the Experiment When you have multiple sets of data, it can be useful to plot them on the same graph for comparison. In the graph here, each set of dots (of the same color) forms a *scatter plot*, in which every data point represents two numerical values, one for each variable. For each data set, a curve that best fits the points has been drawn to make it easier to see the trends. (For additional information about graphs, see the Scientific Skills Review in Appendix F.)

INTERPRET THE DATA

1. First make sure you understand the parts of the graph. (a) Which variable is the independent variable—the variable controlled by the researchers? (b) Which variable is the dependent variable—the variable that depended on the treatment and was measured by the researchers? (c) What do the red dots represent? (d) The blue dots?
2. From the data points on the graph, construct a table of the data. Put "Incubation Time (min)" in the left column of the table.

Glucose Uptake over Time in Guinea Pig Red Blood Cells



Data from T. Kondo and E. Beutler, Developmental changes in glucose transport of guinea pig erythrocytes, *Journal of Clinical Investigation* 65:1–4 (1980).

3. What does the graph show? Compare and contrast glucose uptake in red blood cells from 15-day-old and 1-month-old guinea pigs.
4. Develop a hypothesis to explain the difference between glucose uptake in red blood cells from 15-day-old and 1-month-old guinea pigs. (Think about how glucose gets into cells.)
5. Design an experiment to test your hypothesis.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

No energy input is thus required: This is passive transport. The **Scientific Skills Exercise** gives you an opportunity to work with data from an experiment related to glucose transport.



BioFlix® Animation: Passive Transport

CONCEPT CHECK 8.3

1. How do you think a cell performing cellular respiration rids itself of the resulting CO₂?
2. **WHAT IF? >** If a *Paramecium* swims from a hypotonic to an isotonic environment, will its contractile vacuole become more active or less? Why?

For suggested answers, see Appendix A.

CONCEPT 8.4

Active transport uses energy to move solutes against their gradients

Despite the help of transport proteins, facilitated diffusion is considered passive transport because the solute is moving down its concentration gradient, a process that requires no

energy. Facilitated diffusion speeds transport of a solute by providing efficient passage through the membrane, but it does not alter the direction of transport. Some other transport proteins, however, can move solutes against their concentration gradients, across the plasma membrane from the side where they are less concentrated (whether inside or outside) to the side where they are more concentrated.

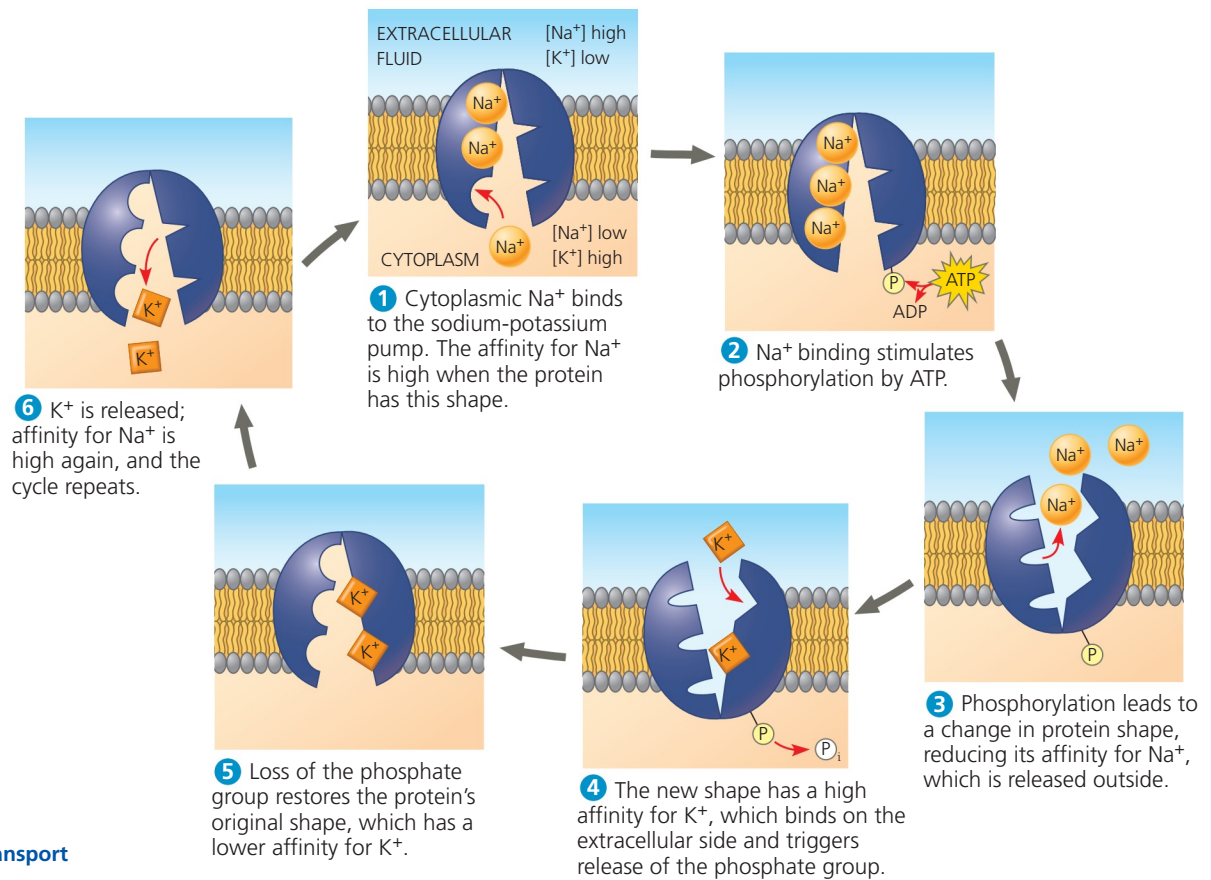
The Need for Energy in Active Transport

To pump a solute across a membrane against its gradient requires work; the cell must expend energy. Therefore, this type of membrane traffic is called **active transport**. The transport proteins that move solutes against their concentration gradients are all carrier proteins rather than channel proteins. This makes sense because when channel proteins are open, they merely allow solutes to diffuse down their concentration gradients rather than picking them up and transporting them against their gradients.

Active transport enables a cell to maintain internal concentrations of small solutes that differ from concentrations in its environment. For example, compared with its

► **Figure 8.15 The sodium-potassium pump: a specific case of active transport.**

This transport system pumps ions against steep concentration gradients: Sodium ion concentration ($[Na^+]$) is high outside the cell and low inside, while potassium ion concentration ($[K^+]$) is low outside the cell and high inside. The pump oscillates between two shapes in a cycle that moves three Na^+ out of the cell (steps 1–3) for every two K^+ pumped into the cell (steps 4–6). The two shapes have different binding affinities for Na^+ and K^+ . ATP hydrolysis powers the shape change by transferring a phosphate group to the transport protein (phosphorylating the protein).



Animation: Active Transport

surroundings, an animal cell has a much higher concentration of potassium ions (K^+) and a much lower concentration of sodium ions (Na^+). The plasma membrane helps maintain these steep gradients by pumping Na^+ out of the cell and K^+ into the cell.

As in other types of cellular work, ATP hydrolysis supplies the energy for most active transport. One way ATP can power active transport is when its terminal phosphate group is transferred directly to the transport protein. This can induce the protein to change its shape in a manner that translocates a solute bound to the protein across the membrane. One transport system that works this way is the **sodium-potassium pump**, which exchanges Na^+ for K^+ across the plasma membrane of animal cells (Figure 8.15). The distinction between passive transport and active transport is reviewed in Figure 8.16.

How Ion Pumps Maintain Membrane Potential

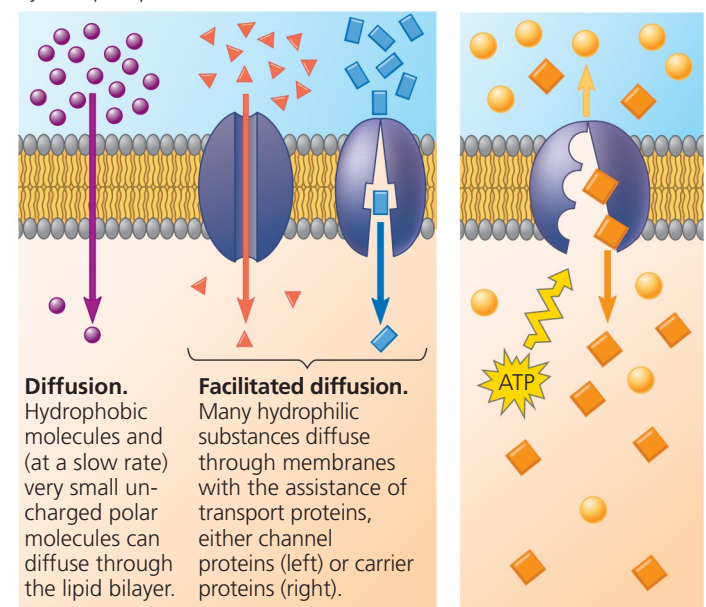
All cells have voltages across their plasma membranes. Voltage is electrical potential energy (see Concept 2.2)—a separation of opposite charges. The cytoplasmic side of the membrane is negative in charge relative to the extracellular side because of an unequal distribution of anions and cations on the two sides. The voltage across a membrane, called a **membrane potential**, ranges from about -50 to -200 millivolts (mV). (The minus sign indicates that the inside of the cell is negative relative to the outside.)

▼ **Figure 8.16 Review: passive and active transport.**

Passive transport. Substances diffuse spontaneously down their concentration gradients, crossing a membrane with no expenditure of energy by the cell. The rate of diffusion can be greatly increased by transport proteins in the membrane.

Active transport.

Some transport proteins act as pumps, moving substances across a membrane against their concentration (or electrochemical) gradients. Energy is usually supplied by ATP hydrolysis.



VISUAL SKILLS ► For each solute in the right panel, describe its direction of movement, and state whether it is moving with or against its concentration gradient.

The membrane potential acts like a battery, an energy source that affects the traffic of all charged substances across the membrane. Because the inside of the cell is negative compared with the outside, the membrane potential favors the passive transport of cations into the cell and anions out of the cell. Thus, *two* forces drive the diffusion of ions across a membrane: a chemical force (the ion's concentration gradient, which has been our sole consideration thus far in the chapter) and an electrical force (the effect of the membrane potential on the ion's movement). This combination of forces acting on an ion is called the **electrochemical gradient**.

In the case of ions, then, we must refine our concept of passive transport: An ion diffuses not simply down its *concentration* gradient but, more exactly, down its *electrochemical* gradient. For example, the concentration of Na^+ inside a resting nerve cell is much lower than outside it. When the cell is stimulated, gated channels open that facilitate Na^+ diffusion. Sodium ions then “fall” down their electrochemical gradient, driven by the concentration gradient of Na^+ and by the attraction of these cations to the negative side (inside) of the membrane. In this example, both electrical and chemical contributions to the electrochemical gradient act in the same direction across the membrane, but this is not always so. In cases where electrical forces due to the membrane potential oppose the simple diffusion of an ion down its concentration gradient, active transport may be necessary. In Concepts 48.2 and 48.3, you'll learn about the importance of electrochemical gradients and membrane potentials in the transmission of nerve impulses.

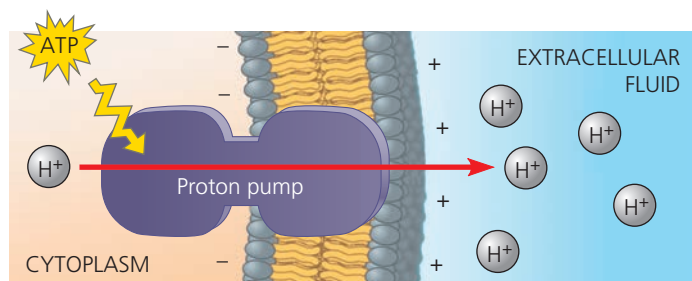
Some membrane proteins that actively transport ions contribute to the membrane potential. An example is the sodium-potassium pump. Notice in Figure 8.15 that the pump does not translocate Na^+ and K^+ one for one, but pumps three sodium ions out of the cell for every two potassium ions it pumps into the cell. With each “crank” of the pump, there is a net transfer of one positive charge from the cytoplasm to the extracellular fluid, a process that stores energy as voltage. A transport protein that generates voltage across a membrane is called an **electrogenic pump**. The sodium-potassium pump appears to be the major

electrogenic pump of animal cells. The main electrogenic pump of plants, fungi, and bacteria is a **proton pump**, which actively transports protons (hydrogen ions, H^+) out of the cell. The pumping of H^+ transfers positive charge from the cytoplasm to the extracellular solution (**Figure 8.17**). By generating voltage across membranes, electrogenic pumps help store energy that can be tapped for cellular work. One important use of proton gradients in the cell is for ATP synthesis during cellular respiration, as you will see in Concept 10.4. Another is a type of membrane traffic called cotransport.

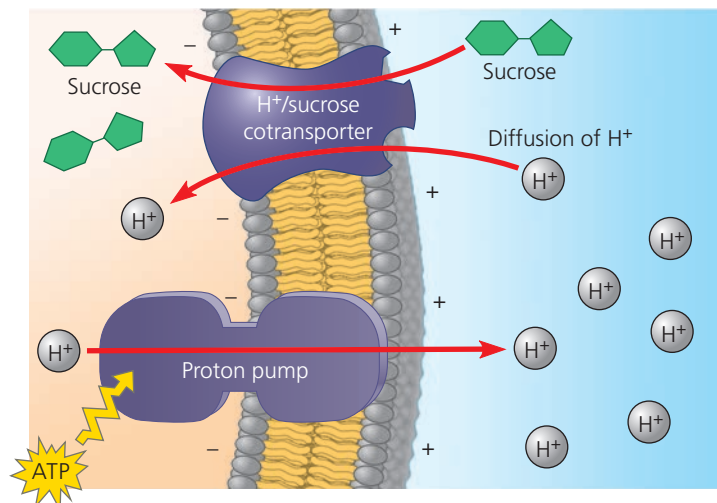
Cotransport: Coupled Transport by a Membrane Protein

A solute that exists in different concentrations across a membrane can do work as it moves across that membrane by diffusion down its concentration gradient. This is analogous to water that has been pumped uphill and performs work as it flows back down. In a mechanism called **cotransport**, a transport protein (a cotransporter) can couple the “downhill” diffusion of the solute to the “uphill” transport of a second substance against its own concentration gradient. For instance, a plant cell uses the gradient of H^+ generated by its ATP-powered proton pumps to drive the active transport of amino acids, sugars, and several other nutrients into the cell. In the example shown in **Figure 8.18**, a cotransporter couples the return of H^+ to the transport of sucrose into the cell. This protein can translocate sucrose into the cell against its concentration gradient, but only if the sucrose molecule travels in the company of an H^+ .

▼ **Figure 8.17 A proton pump.** Proton pumps are electrogenic pumps that store energy by generating voltage (charge separation) across membranes. A proton pump translocates positive charge in the form of hydrogen ions. The voltage and H^+ concentration gradient represent a dual energy source that can drive other processes, such as the uptake of nutrients. Most proton pumps are powered by ATP hydrolysis.



▼ **Figure 8.18 Cotransport: active transport driven by a concentration gradient.** A carrier protein, such as this H^+ /sucrose cotransporter in a plant cell (top), is able to use the diffusion of H^+ down its electrochemical gradient into the cell to drive the uptake of sucrose. (The cell wall is not shown.) Although not technically part of the cotransport process, an ATP-driven proton pump is shown here (bottom), which concentrates H^+ outside the cell. The resulting H^+ gradient represents potential energy that can be used for active transport—of sucrose, in this case. Thus, ATP hydrolysis indirectly provides the energy necessary for cotransport.



The H^+ uses the transport protein as an avenue to diffuse down its own electrochemical gradient, which is maintained by the proton pump. Plants use H^+ /sucrose cotransport to load sucrose produced by photosynthesis into cells in the veins of leaves. The vascular tissue of the plant can then distribute the sugar to roots and other nonphotosynthetic organs that do not make their own food.

What we know about cotransport proteins in animal cells has helped us find more effective treatments for diarrhea, a serious problem in developing countries. Normally, sodium in waste is reabsorbed in the colon, maintaining constant levels in the body, but diarrhea expels waste so rapidly that reabsorption is not possible, and sodium levels fall precipitously. To treat this life-threatening condition, patients are given a solution to drink containing high concentrations of salt (NaCl) and glucose. The solutes are taken up by sodium-glucose cotransporters on the surface of intestinal cells and passed through the cells into the blood. This simple treatment has lowered infant mortality worldwide.



BioFlix® Animation: Active Transport

CONCEPT CHECK 8.4

1. Sodium-potassium pumps help nerve cells establish a voltage across their plasma membranes. Do these pumps use ATP or produce ATP? Explain.
2. **VISUAL SKILLS** > Compare the sodium-potassium pump in Figure 8.15 with the cotransporter in Figure 8.18. Explain why the sodium-potassium pump would not be considered a cotransporter.
3. **MAKE CONNECTIONS** > Review the characteristics of the lysosome in Concept 7.4. Given the internal environment of a lysosome, what transport protein might you expect to see in its membrane?

For suggested answers, see Appendix A.

CONCEPT 8.5

Bulk transport across the plasma membrane occurs by exocytosis and endocytosis

Water and small solutes enter and leave the cell by diffusing through the lipid bilayer of the plasma membrane or by being pumped or moved across the membrane by transport proteins. However, large molecules—such as proteins and polysaccharides, as well as larger particles—generally cross the membrane in bulk, packaged in vesicles. Like active transport, these processes require energy.



BioFlix® Animation: Exocytosis and Endocytosis

Exocytosis

As you saw in Figure 7.15, the cell secretes certain molecules by the fusion of vesicles with the plasma membrane; this process

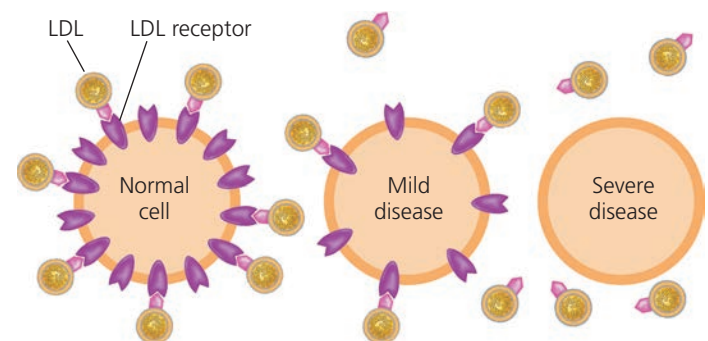
is called **exocytosis**. A transport vesicle that has budded from the Golgi apparatus moves along microtubules of the cytoskeleton to the plasma membrane. When the vesicle membrane and plasma membrane come into contact, specific proteins rearrange the lipid molecules of the two bilayers so that the two membranes fuse. The contents of the vesicle spill out of the cell, and the vesicle membrane becomes part of the plasma membrane (see Figure 8.9, step 4).

Many secretory cells use exocytosis to export products. For example, cells in the pancreas that make insulin secrete it into the extracellular fluid by exocytosis. In another example, nerve cells use exocytosis to release neurotransmitters that signal other neurons or muscle cells. When plant cells are making cell walls, exocytosis delivers some of the necessary proteins and carbohydrates from Golgi vesicles to the outside of the cell.

Endocytosis

In **endocytosis**, the cell takes in molecules and particulate matter by forming new vesicles from the plasma membrane. Although the proteins involved in the processes are different, the events of endocytosis look like the reverse of exocytosis. First, a small area of the plasma membrane sinks inward to form a pocket. Then, as the pocket deepens, it pinches in, forming a vesicle containing material that had been outside the cell. Study **Figure 8.19** carefully to understand the three types of endocytosis: phagocytosis (“cellular eating”), pinocytosis (“cellular drinking”), and receptor-mediated endocytosis.

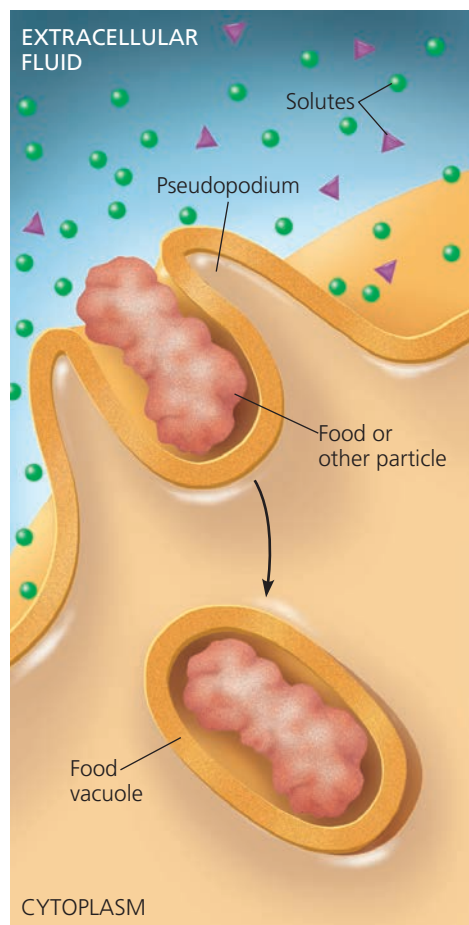
Human cells use receptor-mediated endocytosis to take in cholesterol for membrane synthesis and the synthesis of other steroids. Cholesterol travels in the blood in particles called low-density lipoproteins (LDLs), each a complex of lipids and a protein. LDLs bind to LDL receptors on plasma membranes and then enter the cells by endocytosis. In the inherited disease familial hypercholesterolemia, characterized by a very high level of cholesterol in the blood, LDLs cannot enter cells because the LDL receptor proteins are defective or missing:



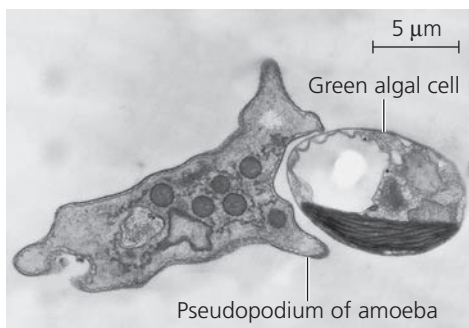
Consequently, cholesterol accumulates in the blood, where it contributes to early atherosclerosis, the buildup of lipid deposits within the walls of blood vessels. This buildup narrows the space in the vessels and impedes blood flow, potentially resulting in heart damage and stroke.

▼ Figure 8.19 Exploring Endocytosis in Animal Cells

Phagocytosis



In **phagocytosis**, a cell engulfs a particle by extending pseudopodia (singular, *pseudopodium*) around it and packaging it within a membranous sac called a food vacuole. The particle will be digested after the food vacuole fuses with a lysosome containing hydrolytic enzymes (see Figure 7.13).

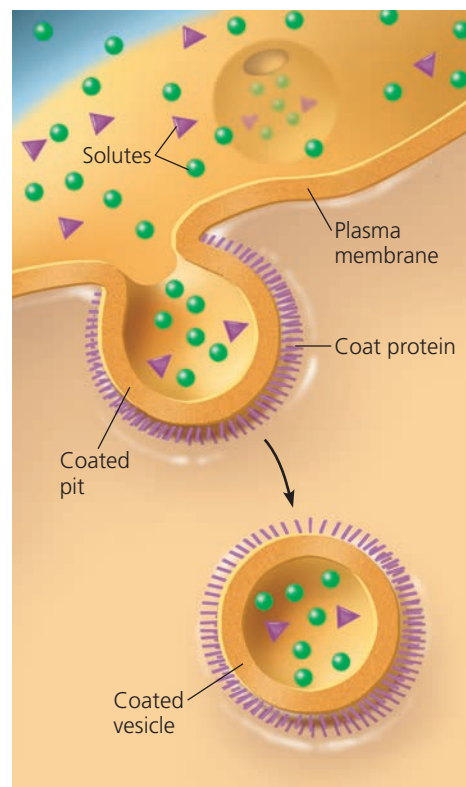


An amoeba engulfing a green algal cell via phagocytosis (TEM).

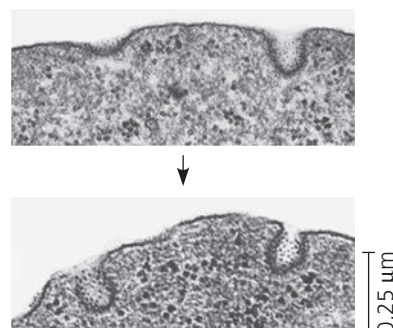
VISUAL SKILLS ► Use the scale bars to estimate the diameters of (a) the food vacuole that will form around the algal cell (left micrograph) and (b) the coated vesicle (lower right micrograph). (c) Which is larger, and by what factor?

Animation: Exocytosis and Endocytosis

Pinocytosis

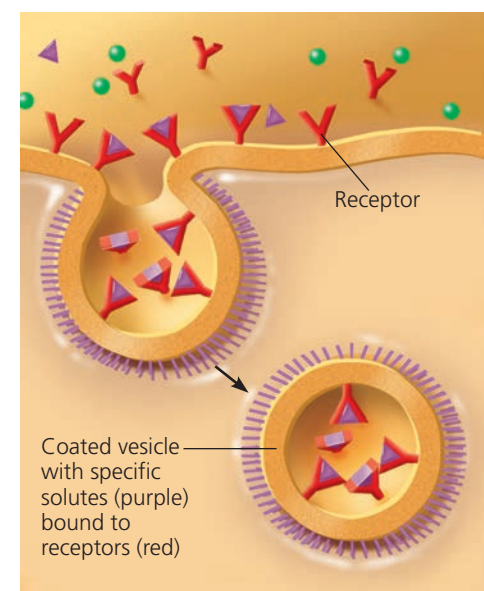


In **pinocytosis**, a cell continually “gulps” droplets of extracellular fluid into tiny vesicles, formed by infoldings of the plasma membrane. In this way, the cell obtains molecules dissolved in the droplets. Because any and all solutes are taken into the cell, pinocytosis as shown here is nonspecific for the substances it transports. In many cases, as above, the parts of the plasma membrane that form vesicles are lined on their cytoplasmic side by a fuzzy layer of coat protein; the “pits” and resulting vesicles are said to be “coated.”

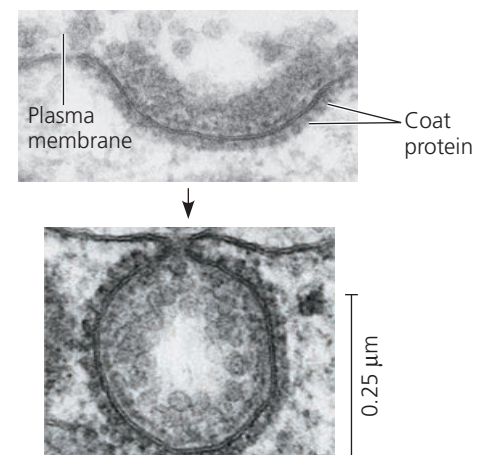


Pinocytotic vesicles forming (TEMs).

Receptor-Mediated Endocytosis



Receptor-mediated endocytosis is a specialized type of pinocytosis that enables the cell to acquire bulk quantities of specific substances, even though those substances may not be very concentrated in the extracellular fluid. Embedded in the plasma membrane are proteins with receptor sites exposed to the extracellular fluid. Specific solutes bind to the receptors. The receptor proteins then cluster in coated pits, and each coated pit forms a vesicle containing the bound molecules. The diagram shows only bound molecules (purple triangles) inside the vesicle, but other molecules from the extracellular fluid are also present. After the ingested material is liberated from the vesicle, the emptied receptors are recycled to the plasma membrane by the same vesicle (not shown).



Top: A coated pit. **Bottom:** A coated vesicle forming during receptor-mediated endocytosis (TEMs).

Endocytosis and exocytosis also provide mechanisms for rejuvenating or remodeling the plasma membrane. These processes occur continually in most eukaryotic cells, yet the amount of plasma membrane in a nongrowing cell remains fairly constant. The addition of membrane by one process appears to offset the loss of membrane by the other.

Energy and cellular work have figured prominently in our study of membranes. In chapters 10 and 11, you will learn more about how cells acquire chemical energy to do the work of life.



BioFlix® Animation: Membrane Transport

CONCEPT CHECK 8.5

1. As a cell grows, its plasma membrane expands. Does this involve endocytosis or exocytosis? Explain.
2. **DRAW IT** ➤ Return to Figure 8.9, and circle a patch of plasma membrane that is coming from a vesicle involved in exocytosis.
3. **MAKE CONNECTIONS** ➤ In Concept 7.7, you learned that animal cells make an extracellular matrix (ECM). Describe the cellular pathway of synthesis and deposition of an ECM glycoprotein.

For suggested answers, see Appendix A.

8 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 8.1

Cellular membranes are fluid mosaics of lipids and proteins (pp. 197–201)

- In the **fluid mosaic model**, **amphipathic** proteins are embedded in the phospholipid bilayer.
- Phospholipids and some proteins move sideways within the membrane. The unsaturated hydrocarbon tails of some phospholipids keep membranes fluid at lower temperatures, while cholesterol helps membranes resist changes in fluidity caused by temperature changes.
- Membrane proteins function in transport, enzymatic activity, signal transduction, cell-cell recognition, intercellular joining, and attachment to the cytoskeleton and extracellular matrix. Short chains of sugars linked to proteins (in **glycoproteins**) and lipids (in **glycolipids**) on the exterior side of the plasma membrane interact with surface molecules of other cells.
- Membrane proteins and lipids are synthesized in the ER and modified in the ER and Golgi apparatus. The inside and outside faces of membranes differ in molecular composition.



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax



In what ways are membranes crucial to life?

CONCEPT 8.2

Membrane structure results in selective permeability (pp. 201–202)

- A cell must exchange molecules and ions with its surroundings, a process controlled by the **selective permeability** of the plasma membrane. Hydrophobic substances are soluble in lipids and pass through membranes rapidly, whereas polar molecules and ions generally require specific **transport proteins**.



How do aquaporins affect the permeability of a membrane?

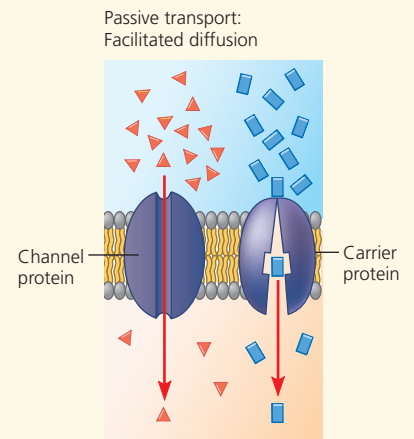
CONCEPT 8.3

Passive transport is diffusion of a substance across a membrane with no energy investment (pp. 202–206)

- **Diffusion** is the spontaneous movement of a substance down its **concentration gradient**. Water diffuses out through the permeable membrane of a cell (**osmosis**) if the solution outside has

a higher solute concentration (**hypertonic**) than the cytosol; water enters the cell if the solution has a lower solute concentration (**hypotonic**). If the concentrations are equal (**isotonic**), no net osmosis occurs. Cell survival depends on balancing water uptake and loss.

- In **facilitated diffusion**, a transport protein speeds the movement of water or a solute across a membrane down its concentration gradient. **Ion channels** facilitate the diffusion of ions across a membrane. Carrier proteins can undergo changes in shape that translocate bound solutes across the membrane.

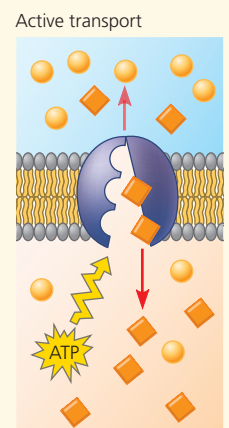


What happens to a cell placed in a hypertonic solution? Describe the free water concentration inside and out.

CONCEPT 8.4

Active transport uses energy to move solutes against their gradients (pp. 206–209)

- Specific membrane proteins use energy, usually in the form of ATP, to do the work of **active transport**.
- Ions can have both a concentration (chemical) gradient and an electrical gradient (voltage). These gradients combine in the **electrochemical gradient**, which determines the net direction of ionic diffusion.
- **Cotransport** of two solutes occurs when a membrane protein enables the “downhill” diffusion of one solute to drive the “uphill” transport of the other.



ATP is not directly involved in the functioning of a cotransporter. Why, then, is cotransport considered active transport?

CONCEPT 8.5

Bulk transport across the plasma membrane occurs by exocytosis and endocytosis (pp. 209–211)

- In **exocytosis**, transport vesicles migrate to the plasma membrane, fuse with it, and release their contents. In **endocytosis**, molecules enter cells within vesicles that pinch inward from the plasma membrane. The three types of endocytosis are **phagocytosis**, **pinocytosis**, and **receptor-mediated endocytosis**.

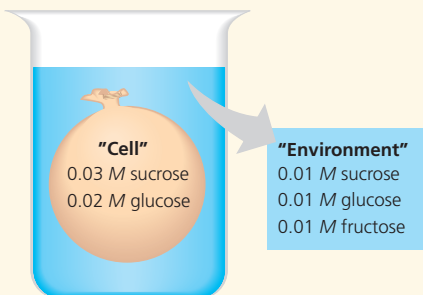
? Which type of endocytosis involves the binding of specific substances in the extracellular fluid to membrane proteins? What does this type of transport enable a cell to do?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

6. **DRAW IT** An artificial “cell” consisting of an aqueous solution enclosed in a selectively permeable membrane is immersed in a beaker containing a different solution, the “environment,” as shown in the accompanying diagram. The membrane is permeable to water and to the simple sugars glucose and fructose but impermeable to the disaccharide sucrose.
- (a) Draw solid arrows to indicate the net movement of solutes into and/or out of the cell.
- (b) Is the solution outside the cell isotonic, hypotonic, or hypertonic?
- (c) Draw a dashed arrow to show the net osmosis, if any.
- (d) Will the artificial cell become more flaccid, more turgid, or stay the same?
- (e) Eventually, will the two solutions have the same or different solute concentrations?



7. **EVOLUTION CONNECTION** *Paramecium* and other unicellular eukaryotes that live in hypotonic environments have cell membranes that limit water uptake, while those living in isotonic environments have membranes that are more permeable to water. Describe what water regulation adaptations might have evolved in unicellular eukaryotes in

hypertonic habitats such as the Great Salt Lake and in habitats with changing salt concentration.

8. **SCIENTIFIC INQUIRY** An experiment is designed to study the mechanism of sucrose uptake by plant cells. Cells are immersed in a sucrose solution, and the pH of the solution is monitored. Samples of the cells are taken at intervals, and their sucrose concentration is measured. The pH is observed to decrease until it reaches a steady, slightly acidic level, and then sucrose uptake begins. (a) Evaluate these results and propose a hypothesis to explain them. (b) Predict what would happen if an inhibitor of ATP regeneration by the cell were added to the beaker once the pH was at a steady level. Explain.
9. **SCIENCE, TECHNOLOGY, AND SOCIETY** Extensive irrigation in arid regions causes salts to accumulate in the soil. (When water evaporates, salts that were dissolved in the water are left behind in the soil.) Based on what you learned about water balance in plant cells, explain why increased soil salinity (saltiness) might be harmful to crops.
10. **WRITE ABOUT A THEME: INTERACTIONS** A human pancreatic cell obtains O_2 —and necessary molecules such as glucose, amino acids, and cholesterol—from its environment, and it releases CO_2 as a waste product. In response to hormonal signals, the cell secretes digestive enzymes. It also regulates its ion concentrations by exchange with its environment. Based on what you have just learned about the structure and function of cellular membranes, write a short essay (100–150 words) to describe how such a cell accomplishes these interactions with its environment.

11. SYNTHESIZE YOUR KNOWLEDGE



In the supermarket, lettuce and other produce are often sprayed with water. Explain why this makes vegetables crisp.

For selected answers, see Appendix A.

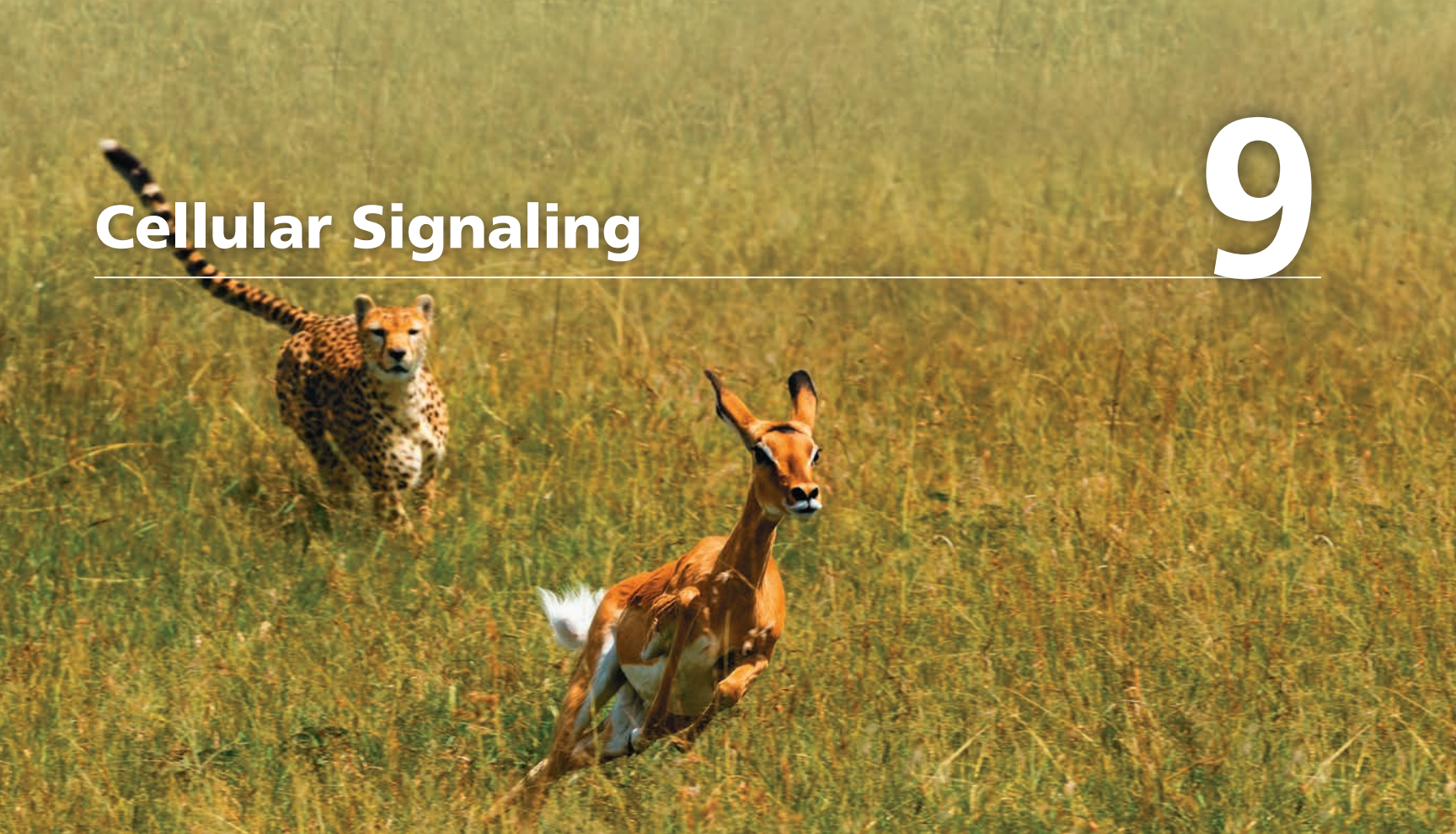


For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

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Cellular Signaling

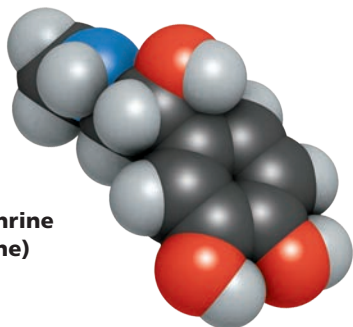
9



▲ **Figure 9.1** How does cell signaling trigger the desperate flight of this impala?

KEY CONCEPTS

- 9.1** External signals are converted to responses within the cell
- 9.2** Reception: A signaling molecule binds to a receptor protein, causing it to change shape
- 9.3** Transduction: Cascades of molecular interactions relay signals from receptors to target molecules in the cell
- 9.4** Response: Cell signaling leads to regulation of transcription or cytoplasmic activities
- 9.5** Apoptosis integrates multiple cell-signaling pathways



► **Epinephrine (adrenaline)**

Cellular Messaging

The impala in **Figure 9.1** flees for its life, racing to escape the predatory cheetah nipping at its heels. The impala is breathing rapidly, its heart pounding and its legs pumping furiously. These physiological functions are all part of the impala's "fight-or-flight" response, driven by hormones released from its adrenal glands at times of stress—in this case, upon sensing the cheetah. What systems allow the trillions of cells in the impala to "talk" to each other, coordinating their activities?

Cells can signal to each other and interpret the signals they receive from other cells and the environment. The signals may include light and touch, but are most often chemicals. The flight response shown here is triggered by a signaling molecule called epinephrine (also called adrenaline; see the space-filling model included here). In studying cell communication, biologists have discovered ample evidence for the evolutionary relatedness of all life. The same set of cell-signaling mechanisms shows up again and again in diverse species, in processes ranging from bacterial signaling to embryonic development to cancer. In this chapter, we focus on the main mechanisms by which cells receive, process, and respond to chemical signals sent from other cells. We will also consider *apoptosis*, a mechanism of programmed cell death that integrates input from multiple signaling pathways.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

CONCEPT 9.1

External signals are converted to responses within the cell

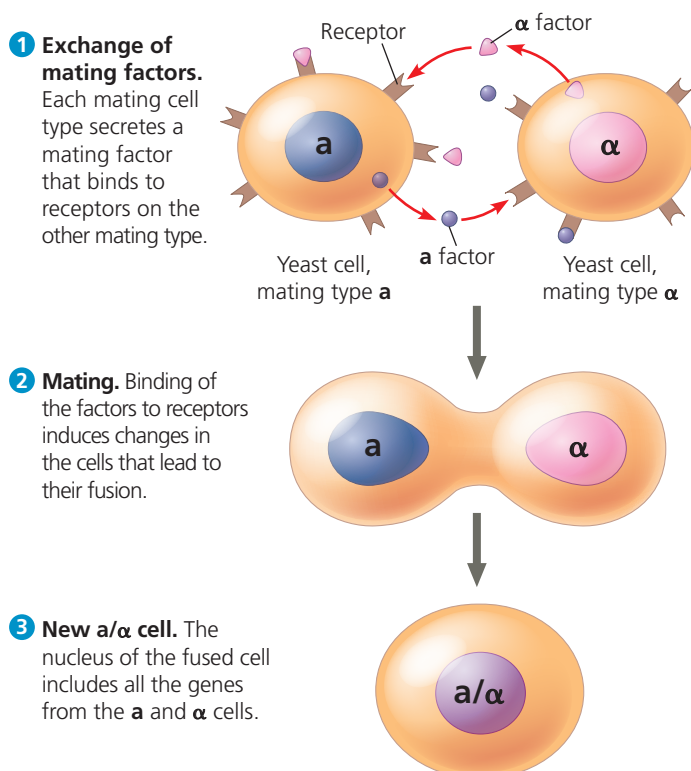
What does a cell that is “talking” say to a “listening” cell, and how does the latter cell respond to the message—that is, how do cells communicate? Let’s approach these questions by first looking at communication among microorganisms.

Evolution of Cell Signaling

EVOLUTION One topic cells communicate about is sex. Cells of the unicellular yeast *Saccharomyces cerevisiae*—which are used to make bread, wine, and beer—identify their sexual mates by chemical signaling. There are two sexes, or mating types, called **a** and **α** (Figure 9.2). Each type secretes a specific factor that binds only to receptors on the other type of cell. When exposed to each other’s mating factors, a pair of cells of opposite type change shape, grow toward each other, and fuse (mate). The new **a/α** cell contains all the genes of both original cells, a combination of genetic resources that provides advantages to the cell’s descendants, which arise by subsequent cell divisions.

The unique match between mating factor and receptor is key to ensuring mating only among cells of the same species of yeast. Recently, researchers were able to genetically engineer yeast cells with both receptors and mating factors altered so

▼ **Figure 9.2 Communication between mating yeast cells.** *Saccharomyces cerevisiae* cells use chemical signaling to identify cells of opposite mating type and initiate the mating process. The two mating types and their corresponding chemical signaling molecules, or mating factors, are called **a** and **α**.



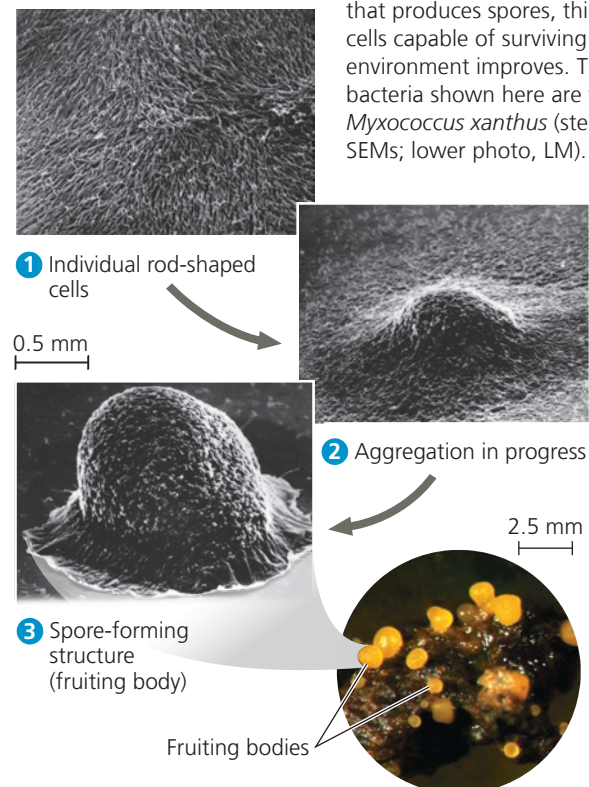
that the altered proteins would bind to each other but not to the original proteins of the parent cells. The genetically engineered cells were thus able to mate with one another but not with cells of the parent population. This evidence supports a model in which changes in the genes encoding receptor and mating factor proteins can lead to the establishment of new species.

How does the binding of a mating factor by the yeast cell surface receptor initiate a signal that brings about the cellular response of mating? This occurs in a series of steps called a *signal transduction pathway*. Many such pathways exist in both yeast and animal cells. In fact, the molecular details of signal transduction in yeasts and mammals are strikingly similar, even though it’s been over a billion years since they shared a common ancestor. This suggests that early versions of cell-signaling mechanisms evolved hundreds of millions of years before the first multicellular creatures appeared on Earth.

Scientists think that signaling mechanisms first evolved in ancient prokaryotes and single-celled eukaryotes like yeasts and then were adopted for new uses by their multicellular descendants. Cell signaling is also critical among prokaryotes. For example, bacterial cells secrete molecules that can be detected by other bacterial cells (Figure 9.3). Sensing the concentration of such signaling molecules allows bacteria to monitor their own local cell density, a phenomenon called *quorum sensing*.

Quorum sensing allows bacterial populations to coordinate their behaviors in activities that require a given number of cells

▼ **Figure 9.3 Communication among bacteria.** Soil-dwelling bacteria called myxobacteria (“slime bacteria”) use chemical signals to share information about nutrient availability. When food is scarce, starving cells secrete a signaling molecule that stimulates neighboring cells to aggregate. The cells form a structure called a fruiting body that produces spores, thick-walled cells capable of surviving until the environment improves. The myxobacteria shown here are the species *Myxococcus xanthus* (steps 1–3, SEMs; lower photo, LM).



PROBLEM-SOLVING EXERCISE

Can a skin wound turn deadly?

"That scrape I got at the game last week looks infected. I wonder if I should go to the doctor?" Contact sports can be hard on your body even if you are in top physical condition. "Contact" in many cases leads to skin wounds that can become infected—and even deadly, if infected with antibiotic-resistant bacteria.

Watch the video in the MasteringBiology Study Area to see what happened when a strain of antibiotic-resistant bacteria called MRSA infected at least one high school student. MRSA stands for methicillin-resistant *Staphylococcus aureus*, a strain of bacteria that is resistant to several types of antibiotics, not just methicillin. Most "staph" infections are not antibiotic-resistant and can be treated with antibiotics.



ABC News Video: MRSA Outbreak

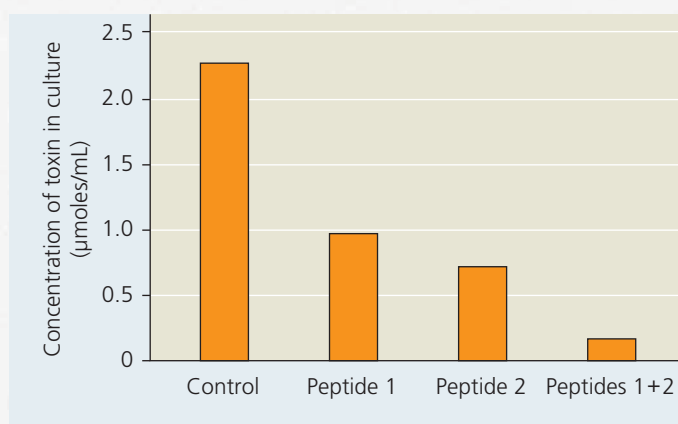
Staphylococcus aureus (*S. aureus*) is a common bacterial species found on the surface of healthy skin that can turn into a serious pathogen if introduced into tissue through a cut or abrasion. Once inside the body, a population of *S. aureus* that reaches a certain density will start to secrete a toxin, killing body cells and contributing significantly to inflammation and damage. Because about 1 in 100 people carry a strain of *S. aureus* that is resistant to common antibiotics, a minor infection can turn permanently harmful or even deadly.

In this exercise, you will investigate the mechanism by which cells sense their own population density (so-called *quorum sensing*) to analyze whether blocking it can stop *S. aureus* from producing toxin.

Your Approach The facts you have in hand for your investigation are that quorum sensing in *S. aureus* involves two separate signal transduction pathways that can lead to toxin production. Two candidate synthetic peptides (short proteins), called peptides 1 and 2, have been proposed to interfere with the *S. aureus* quorum-sensing pathways. Your job is to test these two potential inhibitors of quorum sensing to see if they block either or both of the pathways that lead to toxin production.

For your experiment, you grow four cultures of *S. aureus* to a standardized high density and measure the concentration of toxin in the culture. The control culture contains no peptide. The other cultures have one or both candidate inhibitory peptides mixed into the growth medium before starting the cultures.

Your Data



Data from N. Balaban et al., Treatment of *Staphylococcus aureus* biofilm infection by the quorum-sensing inhibitor RIP, *Antimicrobial Agents and Chemotherapy* 51(6):2226–2229 (2007).

Your Analysis

1. Rank the cultures according to toxin production, from most to least.
2. Which, if any, of the cultures with peptide(s) resulted in a toxin concentration similar to the control culture? What is your evidence for this?
3. Was there an additive effect on toxin production when peptides 1 and 2 were both present in the growth medium? What is your evidence for this?
4. Based on these data, would you hypothesize that peptides 1 and 2 act on the same quorum-sensing pathway leading to toxin production or on two different pathways? What is your reasoning?
5. Do these data suggest a possible treatment for antibiotic-resistant *S. aureus* infections? What else would you want to know to investigate this further?



Instructors: A version of this Problem-Solving Exercise can be assigned in MasteringBiology. Or a more extensive investigation called "Solve It: Is It Possible to Treat Bacterial Infections Without Traditional Antibiotics?" can be assigned.

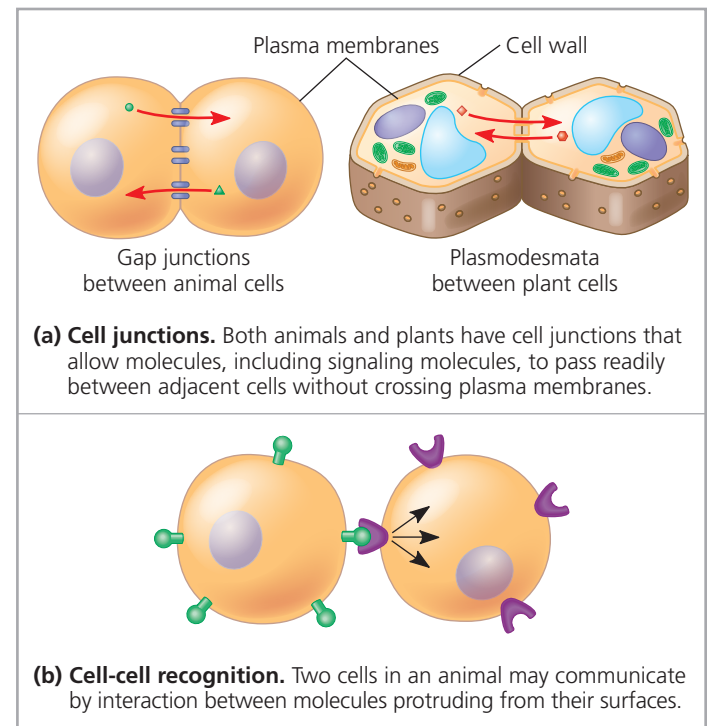
acting synchronously. One example is formation of a *biofilm*, an aggregation of bacterial cells adhered to a surface. The cells in the biofilm often derive nutrition from the surface they are on. You have probably encountered biofilms many times, perhaps without realizing it. The slimy coating on a fallen log or on leaves lying on a forest path, and even the film on your teeth each morning, are examples of bacterial biofilms. In fact, tooth-brushing and flossing disrupt biofilms that would otherwise cause cavities and gum disease.

Another example of bacterial behavior coordinated by quorum sensing is one that has serious medical implications: the secretion of toxins by infectious bacteria. Sometimes treatment by antibiotics doesn't work with such infections due to antibiotic resistance that has evolved in a particular strain of bacteria. Interfering with the signaling pathways used in quorum sensing represents a promising approach as an alternative treatment. In the **Problem-Solving Exercise**, you can participate in the process of scientific thinking involved in this novel approach.

Local and Long-Distance Signaling

Like bacteria or yeast cells, cells in a multicellular organism usually communicate via signaling molecules targeted for cells that may or may not be immediately adjacent. As we saw in Concepts 7.7 and 8.1, eukaryotic cells may communicate by direct contact, which is one type of local signaling (**Figure 9.4**). Both animals and plants have cell junctions that, where present, directly connect the cytoplasms of adjacent cells (**Figure 9.4a**). In these cases, signaling substances dissolved in the cytosol can pass freely between neighboring cells. Moreover, animal cells may communicate via direct contact between membrane-bound cell-surface molecules,

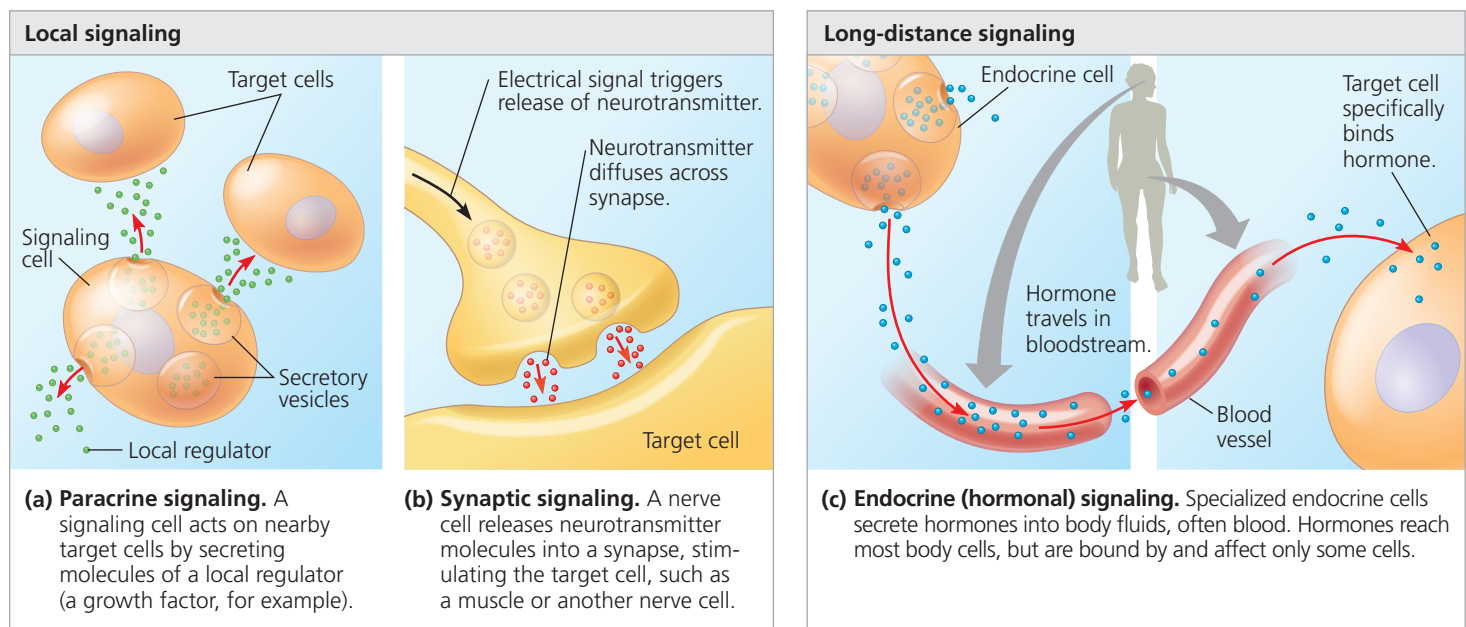
▼ **Figure 9.4 Communication by direct contact between cells.**



in a process called cell-cell recognition (**Figure 9.4b**). This sort of local signaling is especially important in embryonic development and the immune response.

In many other cases of local signaling, signaling molecules are secreted by the signaling cell. Some molecules travel only short distances; such local regulators influence cells in the vicinity. This type of local signaling in animals is called *paracrine signaling* (**Figure 9.5a**). One class of local regulators in

▼ **Figure 9.5 Local and long-distance cell signaling by secreted molecules in animals.** In both local and long-distance signaling, only specific target cells that can recognize a given signaling molecule will respond to it.



animals, *growth factors*, are compounds that stimulate nearby target cells to grow and divide. Numerous cells can simultaneously receive and respond to the growth factors produced by a single cell in their vicinity.

A more specialized type of local signaling called *synaptic signaling* occurs in the animal nervous system (Figure 9.5b). An electrical signal along a nerve cell triggers the secretion of neurotransmitter molecules. These molecules act as chemical signals, diffusing across the synapse—the narrow space between the nerve cell and its target cell—triggering a response in the target cell.

Both animals and plants use molecules called **hormones** for long-distance signaling. In hormonal signaling in animals, also known as *endocrine signaling*, specialized cells release hormones, which travel via the circulatory system to other parts of the body, where they reach target cells that can recognize and respond to them (Figure 9.5c). Plant hormones (often called *plant growth regulators*) sometimes travel in plant vessels (tubes) but more often reach their targets by moving through cells or by diffusing through the air as a gas (see Concept 39.2). Like local regulators, hormones vary widely in size and type. For instance, the plant hormone ethylene, a gas that promotes fruit ripening and helps regulate growth, is a hydrocarbon of only six atoms (C_2H_4), small enough to pass through cell walls. In contrast, the mammalian hormone insulin, which regulates sugar levels in the blood, is a protein with thousands of atoms.

What happens when a potential target cell is exposed to a secreted signaling molecule? The ability of a cell to respond is determined by whether it has a specific receptor molecule that can bind to the signaling molecule. The information conveyed by this binding, the signal, must then be changed into another form—transduced—inside the cell before the cell can respond. The remainder of the chapter discusses this process, primarily as it occurs in animal cells.

The Three Stages of Cell Signaling: A Preview

Our current understanding of how signaling molecules act via signal transduction pathways had its origins in the pioneering work of Earl W. Sutherland, whose research led to a Nobel Prize

in 1971. Sutherland and his colleagues at Vanderbilt University were investigating how the animal hormone epinephrine (also called adrenaline) triggers the “fight-or-flight” response in animals by stimulating the breakdown of the storage polysaccharide glycogen within liver cells and skeletal muscle cells. Glycogen breakdown releases the sugar glucose 1-phosphate, which the cell converts to glucose 6-phosphate. The liver or muscle cell can then use this compound, for energy production in glycolysis (see Chapter 10). Alternatively, the compound can be stripped of phosphate and released from the cell into the blood as glucose, which can fuel cells throughout the body. Thus, one effect of epinephrine is the mobilization of fuel reserves, which can be used by the animal to either defend itself (fight) or escape whatever elicited a scare (flight), as the impala in Figure 9.1 is clearly doing.

Sutherland’s research team discovered that epinephrine stimulates glycogen breakdown by somehow activating a cytosolic enzyme, glycogen phosphorylase. However, when epinephrine was added to a cell-free mixture containing the enzyme and its substrate, glycogen, no breakdown occurred. Glycogen phosphorylase could be activated by epinephrine only when the hormone was added to *intact* cells. This result told Sutherland two things. First, epinephrine does not interact directly with the enzyme responsible for glycogen breakdown; an intermediate step or series of steps must be occurring in the cell. Second, an intact, membrane-bound cell must be present for transmission of the signal to take place.

Sutherland’s work suggested that the process going on at the receiving end of a cellular communication can be dissected into three stages: reception, transduction, and response (Figure 9.6):

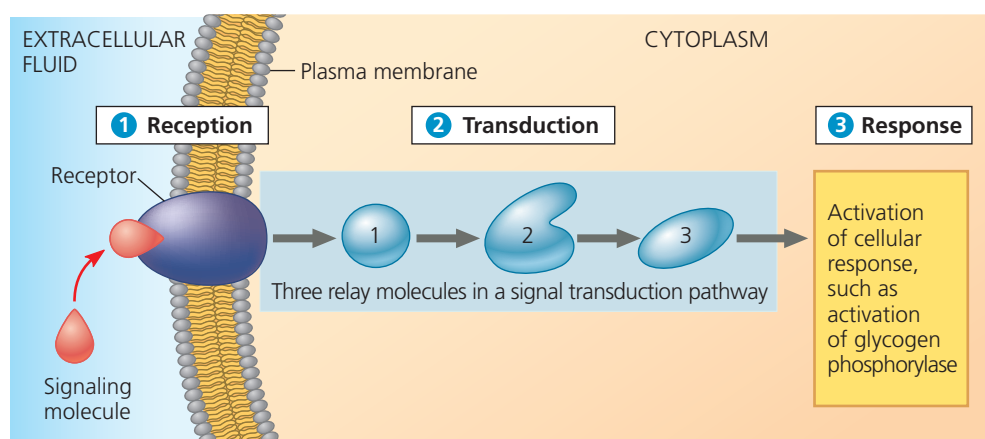
1 Reception. Reception is the target cell’s detection of a signaling molecule coming from outside the cell. A chemical signal is “detected” when the signaling molecule binds to a receptor protein located at the cell’s surface (or inside the cell, to be discussed later).

2 Transduction. The binding of the signaling molecule changes the receptor protein in some way, initiating the

► Figure 9.6 Overview of cell signaling.

From the perspective of the cell receiving the message, cell signaling can be divided into three stages: signal reception, signal transduction, and cellular response. When reception occurs at the plasma membrane, as shown here, the transduction stage is usually a pathway of several steps (three are shown as an example), with each specific relay molecule in the pathway bringing about a change in the next molecule. The final molecule in the pathway triggers the cell’s response.

VISUAL SKILLS ► Where would the epinephrine in Sutherland’s experiment fit into this diagram of cell signaling?



Animation: Overview of Cell Signaling

process of transduction. The transduction stage converts the signal to a form that can bring about a specific cellular response. In Sutherland's system, the binding of epinephrine to a receptor protein in a liver cell's plasma membrane leads to activation of glycogen phosphorylase in the cytosol. Transduction sometimes occurs in a single step but more often requires a sequence of changes in a series of different molecules—a **signal transduction pathway**. The molecules in the pathway are often called relay molecules; three are shown as an example.

3 Response. In the third stage of cell signaling, the transduced signal finally triggers a specific cellular response. The response may be almost any imaginable cellular activity—such as catalysis by an enzyme (for example, glycogen phosphorylase), rearrangement of the cytoskeleton, or activation of specific genes in the nucleus. The cell-signaling process helps ensure that crucial activities like these occur in the right cells, at the right time, and in proper coordination with the activities of other cells of the organism. We'll now explore the mechanisms of cell signaling in more detail, including a discussion of regulation and termination of the process.

CONCEPT CHECK 9.1

1. Explain how signaling is involved in ensuring that yeast cells fuse only with cells of the opposite mating type.
2. How do distantly placed cells in a multicellular organism communicate?
3. **WHAT IF? >** If epinephrine were mixed with glycogen phosphorylase and glycogen in a cell-free mixture in a test tube, would glucose 1-phosphate be generated? Why or why not?

For suggested answers, see Appendix A.

CONCEPT 9.2

Reception: A signaling molecule binds to a receptor protein, causing it to change shape

A wireless router may broadcast its network signal indiscriminately, but often it can be joined only by computers with the correct password: Reception of the signal depends on the receiver. Similarly, the signals emitted by an α mating type yeast cell are “heard” only by its prospective mates, α cells. In the case of the epinephrine circulating throughout the bloodstream of the impala in Figure 9.1, the hormone encounters many types of cells, but only certain target cells detect and react to the epinephrine molecule. A receptor protein on or in the target cell allows the cell to “hear” the signal and respond to it. The signaling molecule is complementary in shape to a specific site on the receptor and attaches there, like a hand in a glove. The signaling molecule

acts as a **ligand**, the term for a molecule that specifically binds to another (often larger) molecule. Ligand binding generally causes a receptor protein to undergo a change in shape. For many receptors, this shape change directly activates the receptor, enabling it to interact with other cellular molecules. For other kinds of receptors, the immediate effect of ligand binding is to cause the aggregation of two or more receptor proteins, which leads to further molecular events inside the cell. Most signal receptors are plasma membrane proteins, but others are located inside the cell. We discuss both of these types next.



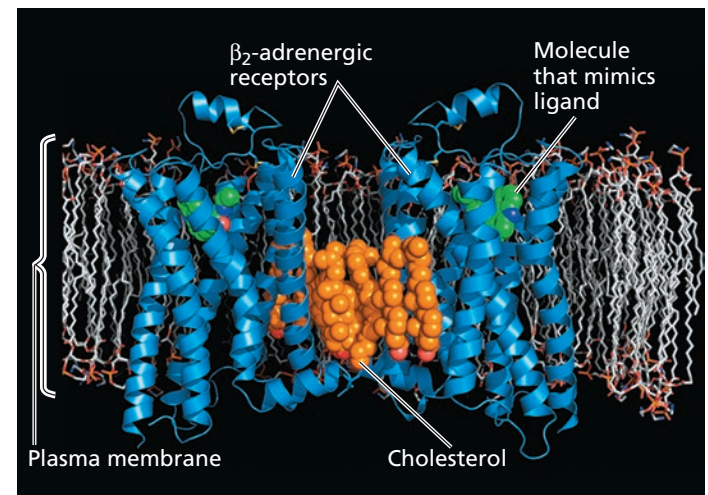
Animation: Reception

Receptors in the Plasma Membrane

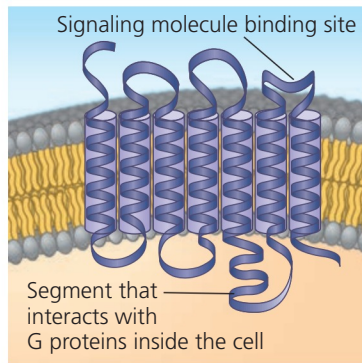
Cell-surface transmembrane receptors play crucial roles in the biological systems of animals. The largest family of human cell-surface receptors is the G protein-coupled receptors (GPCRs). There are more than 800 GPCRs; an example is shown in **Figure 9.7**. Another example is the co-receptor hijacked by HIV to enter immune cells (see Figure 8.8); this GPCR is the target of the drug maraviroc, which has shown some success at treating AIDS.

Most water-soluble signaling molecules bind to specific sites on transmembrane receptor proteins that transmit information from the extracellular environment to the inside of the cell. We can see how cell-surface transmembrane receptors work by looking at three major types: G protein-coupled receptors (GPCRs), receptor tyrosine kinases, and ion channel receptors. These receptors are discussed and illustrated in **Figure 9.8**; study this figure before going on.

✓ Figure 9.7 The structure of a G protein-coupled receptor (GPCR). Shown here is a model of the human β_2 -adrenergic receptor, which binds adrenaline (epinephrine) and was able to be crystallized in the presence of both a molecule that mimics adrenaline (green in the model) and cholesterol in the membrane (orange). Two receptor molecules (blue) are shown as ribbon models in a side view. Caffeine can also bind to this receptor.



G Protein-Coupled Receptors



G protein-coupled receptor

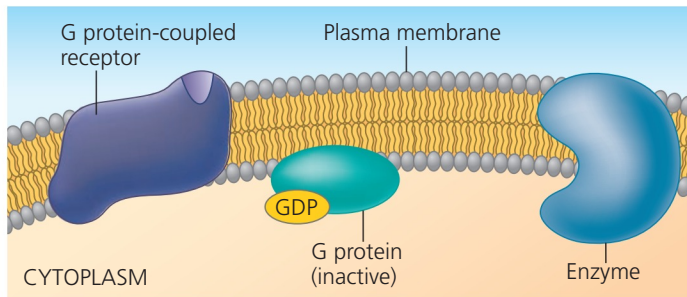
A **G protein-coupled receptor** (GPCR) is a cell-surface transmembrane receptor that works with the help of a **G protein**, a protein that binds the energy-rich molecule GTP. Many different signaling molecules—including yeast mating factors, neurotransmitters, and epinephrine (adrenaline) and many other hormones—use GPCRs.

G protein-coupled receptors vary in the binding sites for their ligands and also for different types of G proteins inside the cell. Nevertheless, GPCR proteins are all remarkably similar in structure. In fact, they make up a large family of eukaryotic receptor proteins with a secondary structure in which the single polypeptide, represented here in a ribbon model, has seven transmembrane α helices (outlined with cylinders and depicted in a row for clarity). Specific loops between the helices (here, the loops on the right) form binding

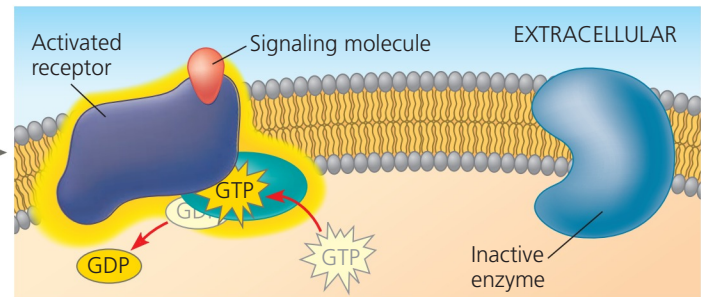
sites for signaling molecules (outside the cell) and G proteins (on the cytoplasmic side).

GPCR-based signaling systems are extremely widespread and diverse in their functions, including roles in embryonic development and sensory reception. In humans, for example, vision, smell, and taste depend on GPCRs (see Concept 50.4). Similarities in structure in G proteins and GPCRs in diverse organisms suggest that G proteins and their associated receptors evolved very early among eukaryotes.

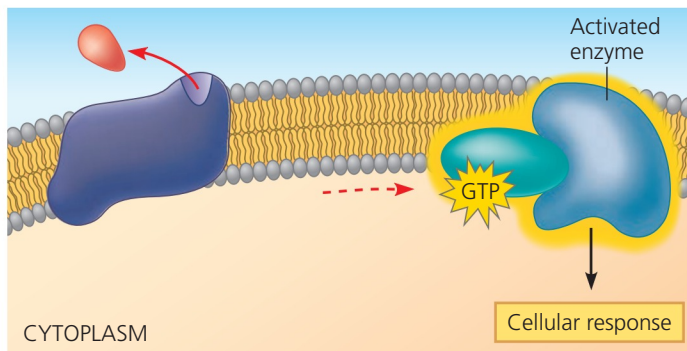
Malfunctions of the associated G proteins themselves are involved in many human diseases, including bacterial infections. The bacteria that cause cholera, pertussis (whooping cough), and botulism, among others, make their victims ill by producing toxins that interfere with G protein function. Up to 60% of all medicines used today exert their effects by influencing G protein pathways.



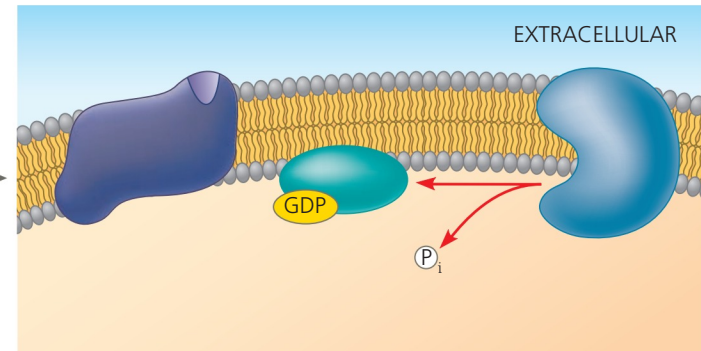
1 Attached but able to move along the cytoplasmic side of the membrane, a G protein functions as a molecular switch that is either on or off, depending on whether GDP or GTP is attached—hence the term *G protein*. (GTP, or guanosine triphosphate, is similar to ATP.) When GDP is bound to the G protein, as shown above, the G protein is inactive. The receptor and G protein work together with another protein, usually an enzyme.



2 When the appropriate signaling molecule binds to the extracellular side of the receptor, the receptor is activated and changes shape. Its cytoplasmic side then binds an inactive G protein, causing a GTP to displace the GDP. This activates the G protein.



3 The activated G protein dissociates from the receptor, diffuses along the membrane, and then binds to an enzyme, altering the enzyme's shape and activity. Once activated, the enzyme can trigger the next step leading to a cellular response. Binding of signaling molecules is reversible: Like other ligands, they bind and dissociate many times. The ligand concentration outside the cell determines how often a ligand is bound and initiates signaling.

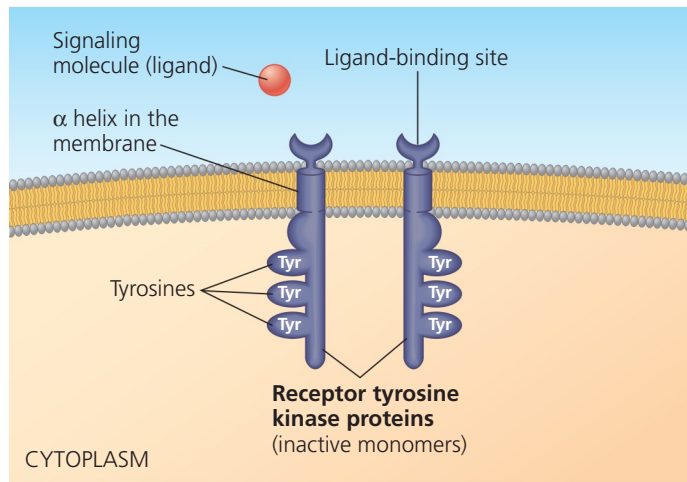


4 The changes in the enzyme and G protein are only temporary because the G protein also functions as a GTPase enzyme—in other words, it then hydrolyzes its bound GTP to GDP and P_i . Now inactive again, the G protein leaves the enzyme, which returns to its original state. The G protein is now available for reuse. The GTPase function of the G protein allows the pathway to shut down rapidly when the signaling molecule is no longer present.

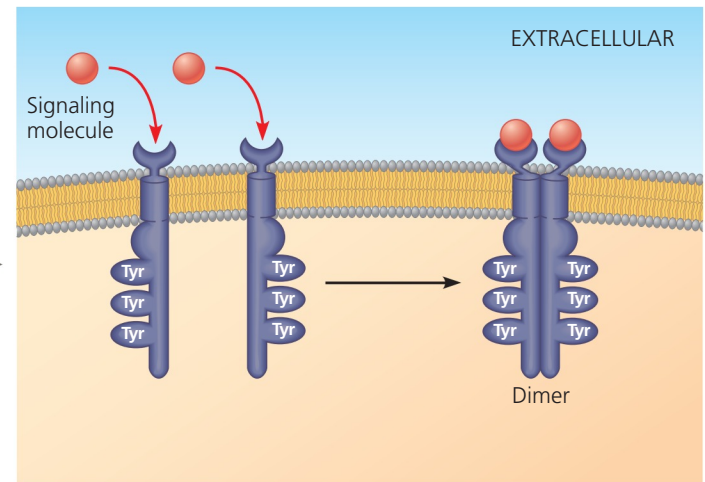
Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) belong to a major class of plasma membrane receptors characterized by having enzymatic activity. An RTK is a *protein kinase*—an enzyme that catalyzes the transfer of phosphate groups from ATP to another protein. The part of the receptor protein extending into the cytoplasm functions more specifically as a tyrosine kinase, an enzyme that catalyzes the transfer of a phosphate group from ATP to the amino acid tyrosine of a substrate protein. Thus, RTKs are membrane receptors that attach phosphates to tyrosines.

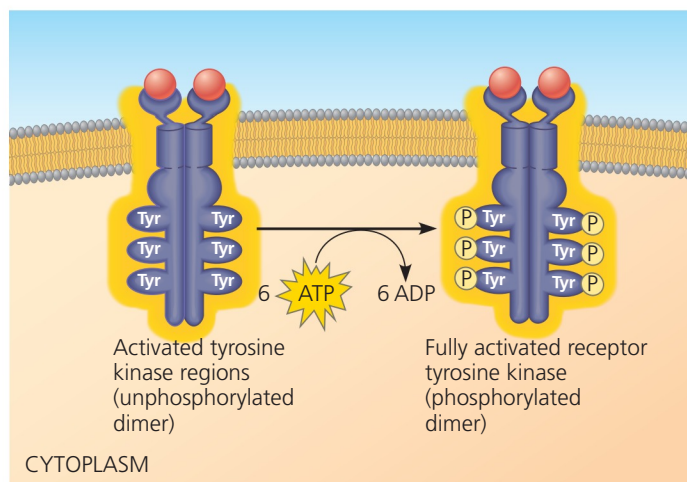
Upon binding a ligand such as a growth factor, one RTK may activate ten or more different transduction pathways and cellular responses. Often, more than one signal transduction pathway can be triggered at once, helping the cell regulate and coordinate many aspects of cell growth and cell reproduction. The ability of a single ligand-binding event to trigger so many pathways is a key difference between RTKs and GPCRs, which generally activate a single transduction pathway. Abnormal RTKs that function even in the absence of signaling molecules are associated with many kinds of cancer.



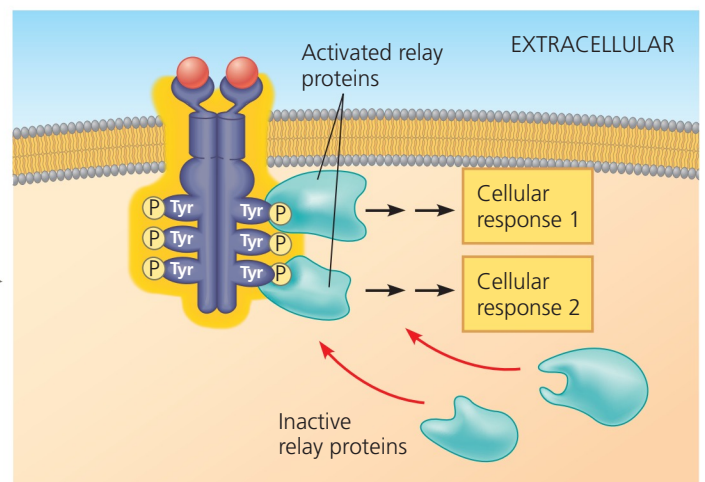
- Many receptor tyrosine kinases have the structure depicted schematically here. Before the signaling molecule binds, the receptors exist as individual units referred to as monomers. Notice that each monomer has an extracellular ligand-binding site, an α helix spanning the membrane, and an intracellular tail containing multiple tyrosines.



- The binding of a signaling molecule (such as a growth factor) causes two receptor monomers to associate closely with each other, forming a complex known as a dimer, in a process called dimerization. (In some cases, larger clusters form. The details of monomer association are a focus of current research.)



- Dimerization activates the tyrosine kinase region of each monomer; each tyrosine kinase adds a phosphate from an ATP molecule to a tyrosine that is part of the tail of the other monomer.



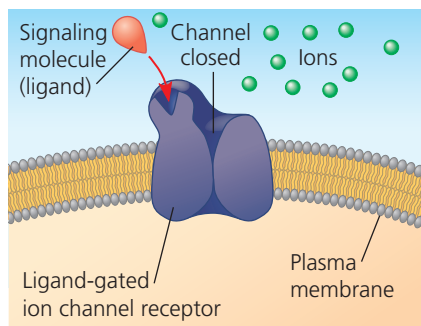
- Now that the receptor is fully activated, it is recognized by specific relay proteins inside the cell. Each such protein binds to a specific phosphorylated tyrosine, undergoing a resulting structural change that activates the bound relay protein. Each activated protein triggers a transduction pathway, leading to a cellular response.

Continued on next page

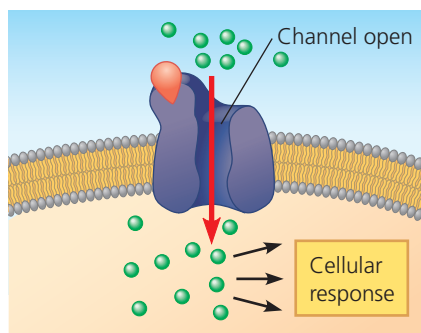
Ion Channel Receptors

A **ligand-gated ion channel** is a type of membrane channel receptor containing a region that can act as a “gate,” opening or closing the channel when the receptor changes shape. When a signaling molecule binds as a ligand to the channel receptor, the channel opens or closes, allowing or blocking the flow of specific ions, such as Na^+ or Ca^{2+} . Like the other receptors we have discussed, these proteins bind the ligand at a specific site on their extracellular sides.

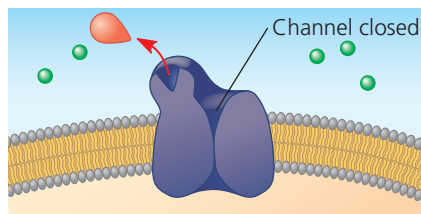
- 1 Here we show a ligand-gated ion channel receptor in which the channel remains closed until a ligand binds to the receptor.



- 2 When the ligand binds to the receptor and the channel opens, specific ions can flow through the channel and rapidly change the concentration of that particular ion inside the cell. This change may directly affect the activity of the cell in some way.



- 3 When the ligand dissociates from this receptor, the channel closes and ions no longer enter the cell.



Ligand-gated ion channels are very important in the nervous system. For example, the neurotransmitter molecules released at a synapse between two nerve cells (see Figure 9.5b) bind as ligands to ion channels on the receiving cell, causing the channels to open. Ions flow in (or, in some cases, out), triggering an electrical signal that propagates down the length of the receiving cell. Some gated ion channels are controlled by electrical signals instead of ligands; these voltage-gated ion channels are also crucial to the functioning of the nervous system, as we will discuss in Chapter 48. Some ion channels are present on membranes of organelles, such as the ER.

MAKE CONNECTIONS ▶ Is the flow of ions through a ligand-gated channel an example of active or passive transport? (Review Concepts 8.3 and 8.4.)



Animation: Acetylcholine Receptor

Given the many important functions of cell-surface receptors, it is not surprising that their malfunctions are associated with many human diseases, including cancer, heart disease, and asthma. To better understand and treat these conditions, a major focus of both university research teams and the pharmaceutical industry has been to analyze the structure of these receptors.

Although cell-surface receptors represent 30% of all human proteins, determining their structures has proved challenging: They make up only 1% of the proteins whose structures have been determined by X-ray crystallography (see Figure 5.21). For one thing, cell-surface receptors tend to be flexible and inherently unstable, thus difficult to crystallize. It took years of persistent efforts for researchers to determine the first few of these structures, such as the GPCR shown in Figure 9.7. In that case, the β -adrenergic receptor was stable enough to be crystallized only while it was among membrane molecules and in the presence of a molecule mimicking its ligand.

Abnormal functioning of receptor tyrosine kinases (RTKs) is associated with many types of cancers. For example, breast cancer patients have a poor prognosis if their tumor cells harbor excessive levels of a receptor tyrosine kinase called HER2 (see the end of Concept 12.3 and Figure 18.27). Using molecular biological techniques, researchers have developed a protein called Herceptin that binds to HER2 on cells and inhibits cell division, thus thwarting further tumor development. In some clinical studies, treatment with Herceptin improved patient survival rates by more than one-third. One goal of ongoing research into these cell-surface receptors and other cell-signaling proteins is development of additional successful treatments.

Intracellular Receptors

Intracellular receptor proteins are found in either the cytoplasm or nucleus of target cells. To reach such a receptor, a signaling molecule passes through the target cell's plasma membrane. A number of important signaling molecules can do this because they are either hydrophobic enough or small enough to cross the hydrophobic interior of the membrane (see Concept 8.1). The hydrophobic signaling molecules include both steroid hormones and thyroid hormones of animals. Another chemical signaling molecule that possesses an intracellular receptor is nitric oxide (NO), a gas; this very small molecule readily passes between the membrane phospholipids. Once a hormone has entered a cell, its binding to an intracellular receptor changes the receptor into a hormone-receptor complex that is able to cause a response—in many cases, the turning on or off of particular genes.

The behavior of aldosterone is a representative example of how steroid hormones work. This hormone is secreted by cells of the adrenal gland, a gland that lies above the kidney. Aldosterone then travels through the blood and enters cells all over the body. However, a response occurs only in kidney cells, which contain receptor molecules

for aldosterone. In these cells, the hormone binds to and activates the receptor protein. With aldosterone attached, the active form of the receptor protein then enters the nucleus and turns on specific genes that control water and sodium flow in kidney cells, ultimately affecting blood volume (Figure 9.9).

How does the activated hormone-receptor complex turn on genes? Recall that the genes in a cell's DNA function by being transcribed and processed into messenger RNA (mRNA), which leaves the nucleus and is translated into a specific protein by ribosomes in the cytoplasm (see Figure 5.22). Special proteins called *transcription factors* control which genes are turned on—that is, which genes are transcribed into mRNA—in a particular cell at a particular time. When the aldosterone receptor is activated, it acts as a transcription factor that turns on specific genes. (You'll learn more about transcription factors in Chapters 17 and 18.)

By acting as a transcription factor, the aldosterone receptor itself carries out the transduction part of the signaling pathway. Most other intracellular receptors function in the same way, although many of them, such as the thyroid hormone receptor, are already in the nucleus before the

signaling molecule reaches them. Interestingly, many of these intracellular receptor proteins are structurally similar, suggesting an evolutionary kinship.

CONCEPT CHECK 9.2

1. Nerve growth factor (NGF) is a water-soluble signaling molecule. Would you expect the receptor for NGF to be intracellular or in the plasma membrane? Why?
2. **WHAT IF? >** What would the effect be if a cell made defective receptor tyrosine kinase proteins that were unable to dimerize?
3. **MAKE CONNECTIONS >** How is ligand binding similar to the process of allosteric regulation of enzymes? (See Figure 6.20.)

For suggested answers, see Appendix A.

CONCEPT 9.3

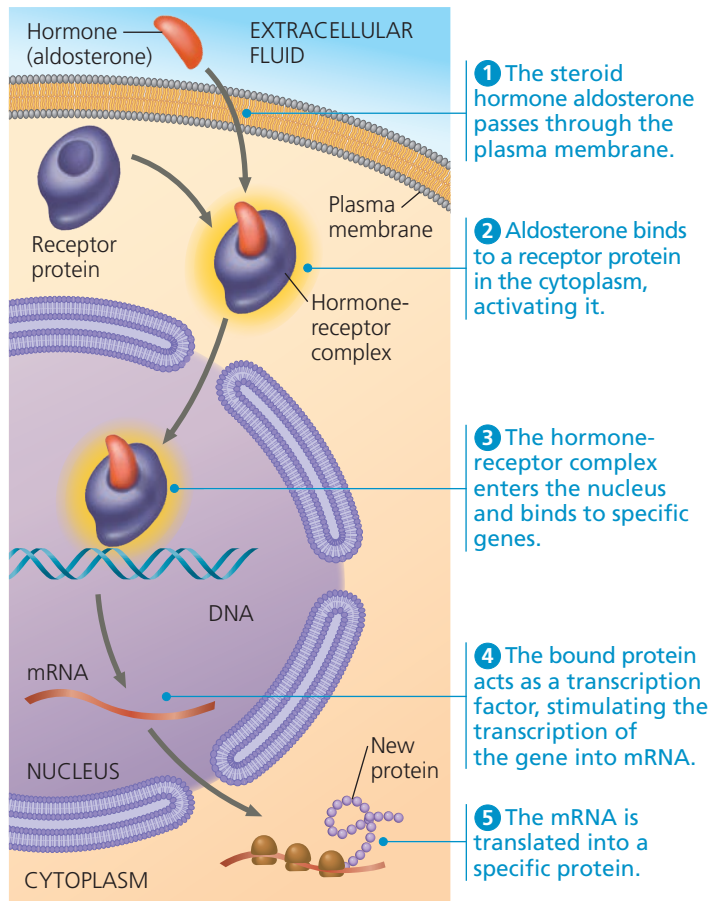
Transduction: Cascades of molecular interactions relay signals from receptors to target molecules in the cell

When receptors for signaling molecules are plasma membrane proteins, like most of those we have discussed, the transduction stage of cell signaling is usually a multistep pathway involving many molecules. Steps often include activation of proteins by addition or removal of phosphate groups or release of other small molecules or ions that act as signaling molecules. One benefit of multiple steps is the possibility of greatly amplifying a signal. If each molecule transmits the signal to numerous molecules at the next step in the series, the result is a geometric increase in the number of activated molecules by the end (see Figure 9.16). Moreover, multistep pathways provide more opportunities for coordination and control than do simpler systems. This allows regulation of the response, as we'll discuss later in the chapter.

Signal Transduction Pathways

The binding of a specific signaling molecule to a receptor in the plasma membrane triggers the first step in the signal transduction pathway—the chain of molecular interactions that leads to a particular response within the cell. Like falling dominoes, the signal-activated receptor activates another molecule, which activates yet another molecule, and so on, until the protein that produces the final cellular response is activated. The molecules that relay a signal from receptor to response, which we call relay molecules in this book, are often proteins. Protein-protein interactions are a major theme of cell signaling—indeed, a unifying theme of all cellular activities.

▼ **Figure 9.9** Steroid hormone interacting with an intracellular receptor.



MAKE CONNECTIONS > Why is a cell-surface receptor protein not required for this steroid hormone to enter the cell? (See Concept 8.2.)



Animation: Signal Transduction Pathways

Keep in mind that the original signaling molecule is not physically passed along a signaling pathway; in most cases, it never even enters the cell. When we say that the signal is relayed along a pathway, we mean that certain information is passed on. At each step, the signal is transduced into a different form, commonly a shape change in the next protein. Very often, the shape change is brought about by phosphorylation.

Protein Phosphorylation and Dephosphorylation

Chapter 6 introduced the concept of activating a protein by adding one or more phosphate groups to it (see Figure 6.11a). In Figure 9.8, you have already seen how phosphorylation is involved in the activation of receptor tyrosine kinases. In fact, the phosphorylation and dephosphorylation of proteins is a widespread cellular mechanism for regulating protein activity. An enzyme that transfers phosphate groups from ATP to a protein is generally known as a **protein kinase**. Recall that a receptor tyrosine kinase is a specific kind of protein kinase that phosphorylates tyrosines on the other receptor tyrosine kinase in a dimer. Most cytoplasmic protein kinases, however, act on proteins different from themselves. Another distinction is that most cytoplasmic protein kinases

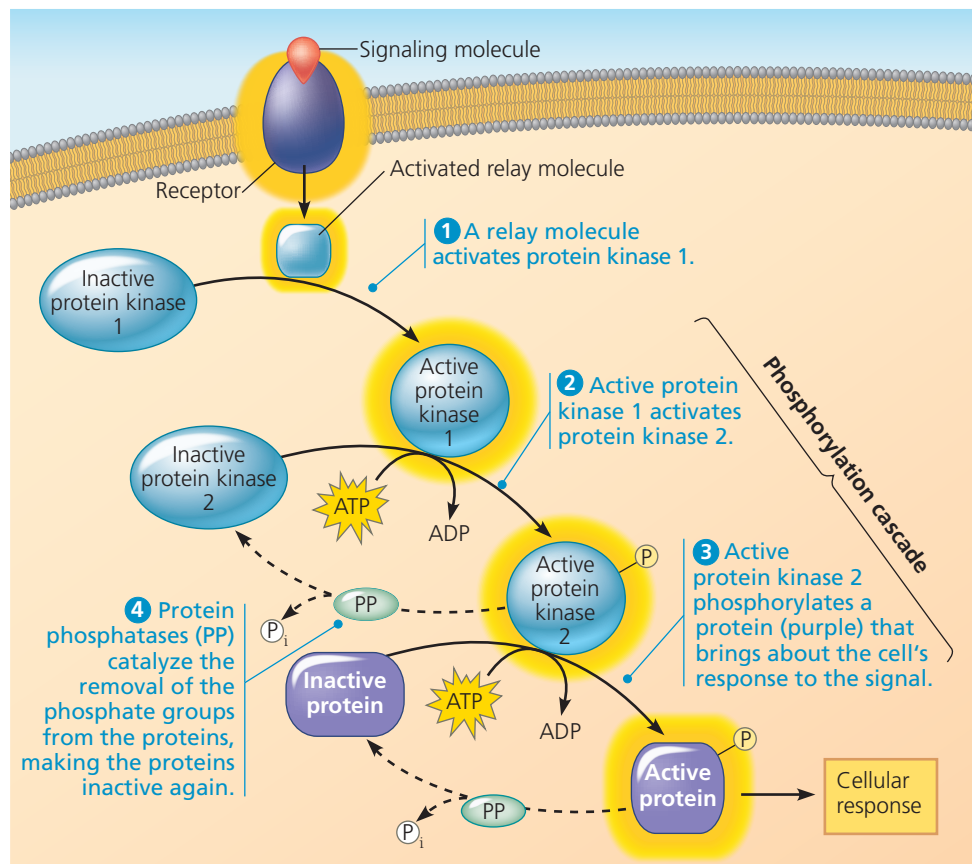
phosphorylate either of two other amino acids, serine or threonine, rather than tyrosine. Serine/threonine kinases are widely involved in signaling pathways in animals, plants, and fungi.

Many of the relay molecules in signal transduction pathways are protein kinases, and they often act on other protein kinases in the pathway. **Figure 9.10** depicts a hypothetical pathway containing two different protein kinases that create a **phosphorylation cascade**. The sequence of steps shown in the figure is similar to many known pathways, including those triggered in yeast by mating factors and in animal cells by many growth factors. The signal is transmitted by a cascade of protein phosphorylations, each causing a shape change in the phosphorylated protein. The shape change results from the interaction of the newly added phosphate groups with charged or polar amino acids on the protein being phosphorylated (see Figure 5.14). The shape change in turn alters the function of the protein, most often activating it. In some cases, though, phosphorylation instead *decreases* the activity of the protein.

About 2% of our own genes are thought to code for protein kinases, a significant percentage. A single cell may have hundreds of different kinds, each specific for a different substrate protein. Together, protein kinases probably regulate the activity of a large proportion of the thousands of proteins in a cell.

► **Figure 9.10 A phosphorylation cascade.** In a phosphorylation cascade, a series of different proteins in a pathway are phosphorylated in turn, each protein adding a phosphate group to the next one in line. Here, phosphorylation activates each protein, and dephosphorylation returns it to its inactive form. The active and inactive forms of each protein are represented by different shapes to remind you that activation is usually associated with a change in molecular shape.

WHAT IF? ► What would happen if a mutation in protein kinase 2 made it incapable of being phosphorylated?



Among these are most of the proteins that, in turn, regulate cell division. Abnormal activity of such a kinase can cause abnormal cell division and contribute to the development of cancer.

Equally important in the phosphorylation cascade are the **protein phosphatases**, enzymes that can rapidly remove phosphate groups from proteins, a process called dephosphorylation. By dephosphorylating and thus inactivating protein kinases, phosphatases provide the mechanism for turning off the signal transduction pathway when the initial signal is no longer present. Phosphatases also make the protein kinases available for reuse, enabling the cell to respond again to an extracellular signal. The phosphorylation-dephosphorylation system acts as a molecular switch in the cell, turning activities on or off, or up or down, as required. At any given moment, the activity of a protein regulated by phosphorylation depends on the balance in the cell between active kinase molecules and active phosphatase molecules.

Small Molecules and Ions as Second Messengers

Not all components of signal transduction pathways are proteins. Many signaling pathways also involve small, non-protein, water-soluble molecules or ions called **second messengers**. (The pathway's "first messenger" is considered to be the extracellular signaling molecule—the ligand—that binds to the membrane receptor.) Because second messengers are small and also water-soluble, they can readily spread throughout the cell by diffusion. For example, as we'll see shortly, a second messenger called cyclic AMP carries the signal initiated by epinephrine from the plasma membrane of a liver or muscle cell into the cell's interior, where the signal eventually brings about glycogen breakdown. Second messengers participate in pathways that are initiated by both G protein-coupled receptors and receptor tyrosine kinases. The two most widely used second messengers are cyclic AMP and calcium ions, Ca^{2+} . A large variety of relay proteins are

sensitive to changes in the cytosolic concentration of one or the other of these second messengers.

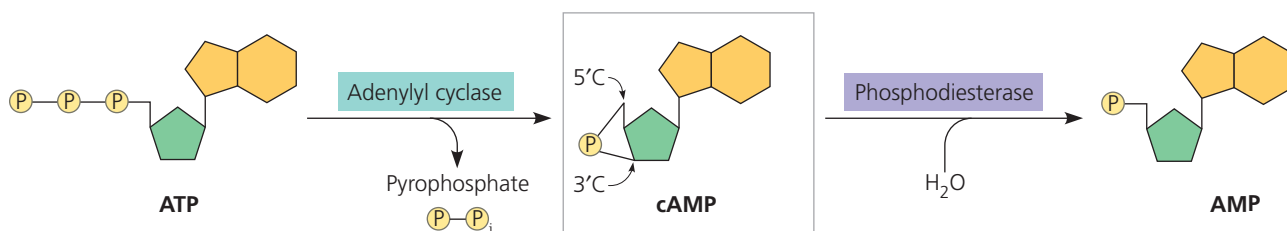
Cyclic AMP

As discussed previously, Earl Sutherland established that, without passing through the plasma membrane, epinephrine somehow causes glycogen breakdown within cells. This discovery prompted him to search for a second messenger that transmits the signal from the plasma membrane to the metabolic machinery in the cytoplasm.

Sutherland found that the binding of epinephrine to the plasma membrane of a liver cell elevates the cytosolic concentration of **cyclic AMP (cAMP)**; cyclic adenosine monophosphate). As shown in **Figure 9.11**, an enzyme embedded in the plasma membrane, **adenylyl cyclase** (also known as adenylyate cyclase), converts ATP to cAMP in response to an extracellular signal—in this case, provided by epinephrine. But epinephrine doesn't stimulate adenylyl cyclase directly. When epinephrine outside the cell binds to a G protein-coupled receptor, the protein activates adenylyl cyclase, which in turn can catalyze the synthesis of many molecules of cAMP. In this way, the normal cellular concentration of cAMP can be boosted 20-fold in a matter of seconds. The cAMP broadcasts the signal to the cytoplasm. It does not persist for long in the absence of the hormone because a different enzyme, called phosphodiesterase, converts cAMP to AMP. Another surge of epinephrine is needed to boost the cytosolic concentration of cAMP again.

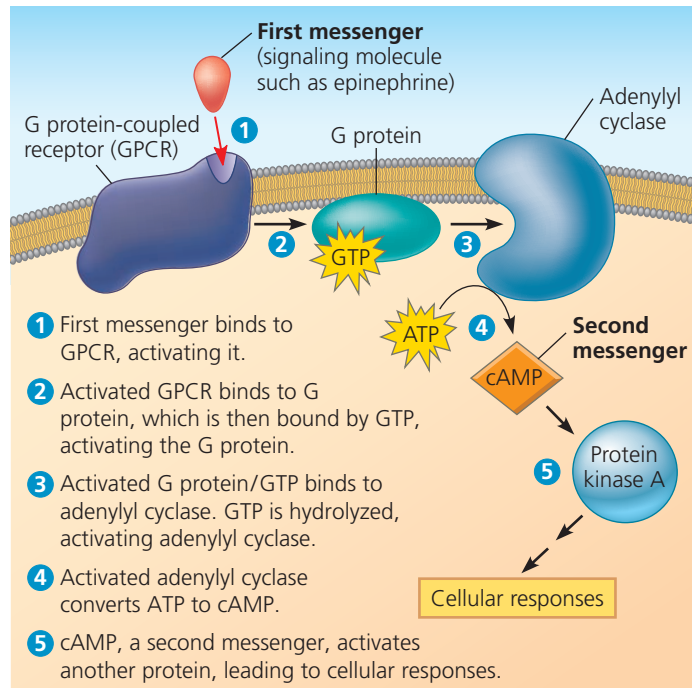
Subsequent research has revealed that epinephrine and many other signaling molecules lead to activation of adenylyl cyclase by G proteins and formation of cAMP (**Figure 9.12**). The immediate effect of an elevation in cAMP levels is usually the activation of a serine/threonine kinase called *protein kinase A*. The activated protein kinase A then phosphorylates various other proteins, depending on the cell type. (The complete pathway for epinephrine's stimulation of glycogen breakdown is shown later, in **Figure 9.16**.)

▼ **Figure 9.11 Cyclic AMP.** The second messenger cyclic AMP (cAMP) is made from ATP by adenylyl cyclase, an enzyme embedded in the plasma membrane. Note that the phosphate group in cAMP is attached to both the 5' and the 3' carbons; this cyclic arrangement is the basis for the molecule's name. Cyclic AMP is inactivated by phosphodiesterase, an enzyme that converts it to AMP.



WHAT IF? > What would happen if a molecule that inactivated phosphodiesterase were introduced into the cell?

▼ **Figure 9.12 cAMP as a second messenger in a G protein signaling pathway.**



DRAW IT ► The bacterium that causes the disease cholera produces a toxin that locks the G protein in its activated state. Review Figure 9.8 then draw this figure as it would be if cholera toxin were present. (You do not need to draw the cholera toxin molecule.)

Further regulation of cell metabolism is provided by other G protein systems that *inhibit* adenylyl cyclase. In these systems, a different signaling molecule activates a different receptor, which in turn activates an *inhibitory* G protein that blocks activation of adenylyl cyclase.

Now that we know about the role of cAMP in G protein signaling pathways, we can explain in molecular detail how certain microbes cause disease. Consider cholera, a disease that is frequently epidemic in places where the water supply is contaminated with human feces. People acquire the cholera bacterium, *Vibrio cholerae*, by drinking contaminated water. The bacteria form a biofilm on the lining of the small intestine and produce a toxin. The cholera toxin is an enzyme that chemically modifies a G protein involved in regulating salt and water secretion. Because the modified G protein is unable to hydrolyze GTP to GDP, it remains stuck in its active form, continuously stimulating adenylyl cyclase to make cAMP (see the question with Figure 9.12). The resulting high concentration of cAMP causes the intestinal cells to secrete large amounts of salts into the intestines, with water following by osmosis. An infected person quickly develops profuse diarrhea and if left untreated can soon die from the loss of water and salts.

Our understanding of signaling pathways involving cyclic AMP or related messengers has allowed us to develop treatments for certain conditions in humans. In one pathway, a molecule similar to cAMP called *cyclic GMP* (cGMP) is produced by a muscle cell in response to the gas nitric oxide (NO) after it is released by a neighboring cell. cGMP then acts as a second messenger that causes relaxation of muscles, such as those in the walls of arteries. A compound that inhibits the hydrolysis of cGMP to GMP, thus prolonging the signal, was originally prescribed for chest pains because it relaxed blood vessels and increased blood flow to the heart muscle. Under the trade name Viagra, this compound is now widely used as a treatment for erectile dysfunction in human males. Because Viagra leads to dilation of blood vessels, it also allows increased blood flow to the penis, optimizing physiological conditions for penile erections.

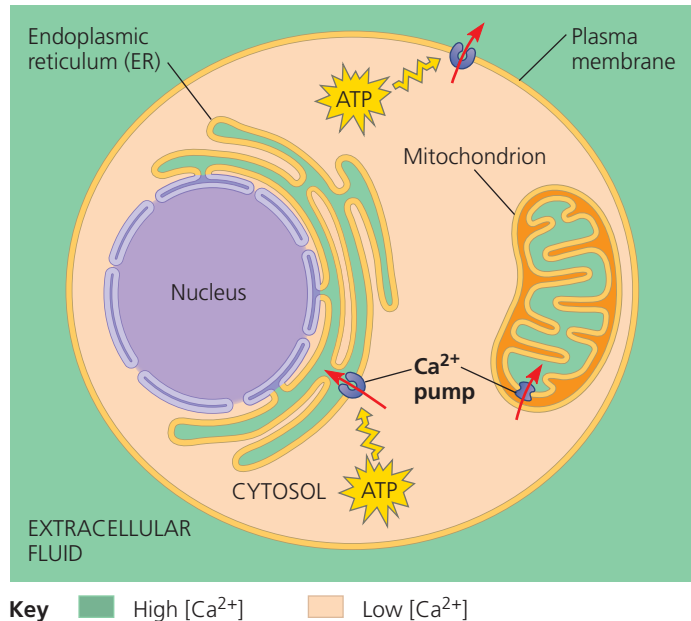
Calcium Ions and Inositol Trisphosphate (IP_3)

Many of the signaling molecules that function in animals—including neurotransmitters, growth factors, and some hormones—induce responses in their target cells via signal transduction pathways that increase the cytosolic concentration of calcium ions (Ca^{2+}). Calcium is even more widely used than cAMP as a second messenger. Increasing the cytosolic concentration of Ca^{2+} causes many responses in animal cells, including muscle cell contraction, exocytosis of molecules (secretion), and cell division. In plant cells, a wide range of hormonal and environmental stimuli can cause brief increases in cytosolic Ca^{2+} concentration, triggering various signaling pathways, such as the pathway for greening in response to light (see Figure 39.4). Cells use Ca^{2+} as a second messenger in pathways triggered by both G protein-coupled receptors and receptor tyrosine kinases.

Although cells always contain some Ca^{2+} , this ion can function as a second messenger because its concentration in the cytosol is normally much lower than the concentration outside the cell (**Figure 9.13**). In fact, the level of Ca^{2+} in the blood and extracellular fluid of an animal is often more than 10,000 times higher than that in the cytosol. Calcium ions are actively transported out of the cell and are actively imported from the cytosol into the endoplasmic reticulum (and, under some conditions, into mitochondria and chloroplasts) by various protein pumps. As a result, the calcium concentration in the ER is usually much higher than that in the cytosol. Because the cytosolic calcium level is low, a small change in absolute numbers of ions represents a relatively large percentage change in calcium concentration.

In response to a signal relayed by a signal transduction pathway, the cytosolic calcium level may rise, usually by a mechanism that releases Ca^{2+} from the cell's ER. The pathways leading to calcium release involve two other second

▼ **Figure 9.13 The maintenance of calcium ion concentrations in an animal cell.** The Ca^{2+} concentration in the cytosol is usually much lower (beige) than in the extracellular fluid and ER (green). Protein pumps in the plasma membrane and the ER membrane, driven by ATP, move Ca^{2+} from the cytosol into the extracellular fluid and into the lumen of the ER. Mitochondrial pumps, driven by chemiosmosis (see Concept 10.4), move Ca^{2+} into mitochondria when the calcium level in the cytosol rises significantly.



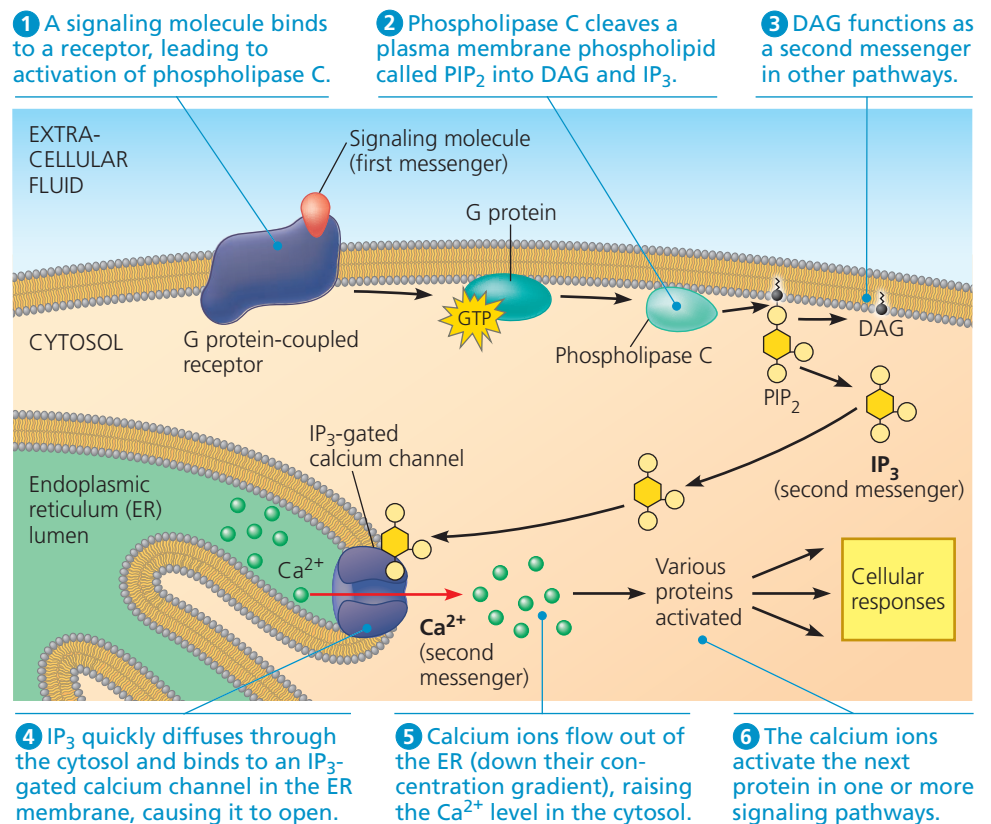
messengers, **inositol trisphosphate (IP_3)** and **diacylglycerol (DAG)**. These two messengers are produced by cleavage of a certain kind of phospholipid in the plasma membrane. **Figure 9.14** shows the complete picture of how a signal causes IP_3 to stimulate the release of calcium from the ER. Because IP_3 acts before calcium in these pathways, calcium could be considered a “third messenger.” However, scientists use the term *second messenger* for all small, nonprotein components of signal transduction pathways.

CONCEPT CHECK 9.3

1. What is a protein kinase, and what is its role in a signal transduction pathway?
2. When a signal transduction pathway involves a phosphorylation cascade, how does the cell’s response get turned off?
3. What is the actual “signal” that is being transduced in any signal transduction pathway, such as those shown in Figures 9.6 and 9.10? In what way is this information being passed from the exterior to the interior of the cell?
4. **WHAT IF? >** If you exposed a cell to a ligand that binds to a receptor and activates phospholipase C, predict the effect the IP_3 -gated calcium channel would have on Ca^{2+} concentration in the cytosol.

For suggested answers, see Appendix A.

► **Figure 9.14 Calcium and IP_3 in signaling pathways.** Calcium ions (Ca^{2+}) and inositol trisphosphate (IP_3) function as second messengers in many signal transduction pathways. In this figure, the process is initiated by the binding of a signaling molecule to a G protein-coupled receptor. A receptor tyrosine kinase could also initiate this pathway by activating phospholipase C.



CONCEPT 9.4

Response: Cell signaling leads to regulation of transcription or cytoplasmic activities

We now take a closer look at the cell's subsequent response to an extracellular signal—what some researchers call the “output response.” What is the nature of the final step in a signaling pathway?

Nuclear and Cytoplasmic Responses

Ultimately, a signal transduction pathway leads to the regulation of one or more cellular activities. The response at the end of the pathway may occur in the nucleus of the cell or in the cytoplasm.

Many signaling pathways ultimately regulate protein synthesis, usually by turning specific genes on or off in the nucleus. Like an activated steroid receptor (see Figure 9.9), the final activated molecule in a signaling pathway may function as a transcription factor. **Figure 9.15** shows an example in which a signaling pathway activates a transcription factor that turns a gene on: The response to this growth factor signal is transcription, the synthesis of one or more specific mRNAs, which will be translated in the cytoplasm into specific proteins. In other cases, the transcription factor might regulate a gene by turning it off. Often a transcription factor regulates several different genes.

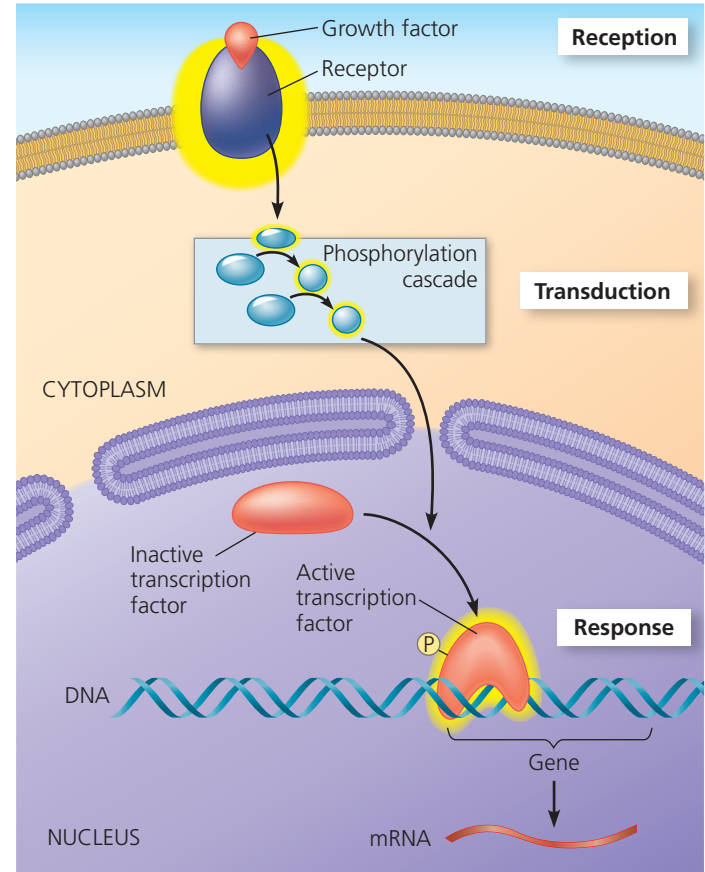
Sometimes a signaling pathway may regulate the *activity* of proteins rather than causing their *synthesis* by activating gene expression. This directly affects proteins that function outside the nucleus. For example, a signal may cause the opening or closing of an ion channel in the plasma membrane or a change in the activity of a metabolic enzyme. As we have seen, the response of liver cells to the hormone epinephrine helps regulate cellular energy metabolism by affecting the activity of an enzyme. The final step in the signaling pathway that begins with epinephrine binding activates the enzyme that catalyzes the breakdown of glycogen. **Figure 9.16** shows the complete pathway leading to the release of glucose 1-phosphate molecules from glycogen. Notice that as each molecule is activated, the response is amplified, a subject we'll return to shortly.

Signal receptors, relay molecules, and second messengers participate in a variety of pathways, leading to both nuclear and cytoplasmic responses, including cell division. Malfunctioning of growth factor pathways like the one in Figure 9.15 can contribute to abnormal cell division and the development of cancer, as we'll see in Concept 18.5.

Regulation of the Response

Whether the response occurs in the nucleus or in the cytoplasm, it is not simply turned “on” or “off.” Rather, the extent

▼ **Figure 9.15 Nuclear responses to a signal: the activation of a specific gene by a growth factor.** This diagram shows a typical signaling pathway that leads to regulation of gene activity in the cell nucleus. The initial signaling molecule, in this case a growth factor, triggers a phosphorylation cascade, as in Figure 9.10. (The ATP molecules and phosphate groups are not shown.) Once phosphorylated, the last kinase in the sequence enters the nucleus and activates a transcription factor, which stimulates transcription of a specific gene (or genes). The resulting mRNAs then direct the synthesis of a particular protein.



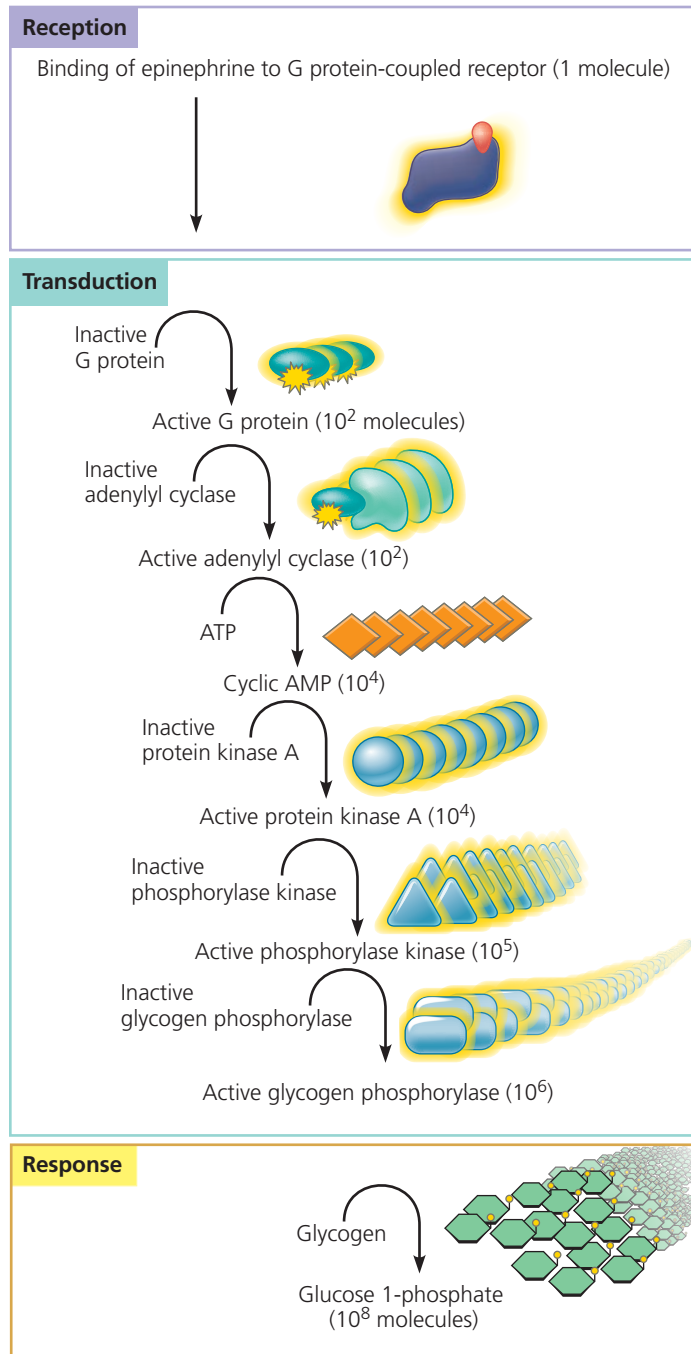
Animation: Nuclear Response: Activating a Gene

and specificity of the response are regulated in multiple ways. Here we'll consider four aspects of this regulation. First, as mentioned earlier, signaling pathways generally amplify the cell's response to a single signaling event. The degree of amplification depends on the function of the specific molecules in the pathway. Second, the many steps in a multistep pathway provide control points at which the cell's response can be further regulated, contributing to the specificity of the response and allowing coordination with other signaling pathways. Third, the overall efficiency of the response is enhanced by the presence of proteins known as scaffolding proteins. Finally, a crucial point in regulating the response is the termination of the signal.

Signal Amplification

Elaborate enzyme cascades amplify the cell's response to a signal. At each catalytic step in the cascade, the number

▼ **Figure 9.16 Cytoplasmic response to a signal: the stimulation of glycogen breakdown by epinephrine (adrenaline).** In this signaling system, the hormone epinephrine acts through a G protein-coupled receptor to activate a succession of relay molecules, including cAMP and two protein kinases (see also Figure 9.12). The final protein activated is the enzyme glycogen phosphorylase, which uses inorganic phosphate to release glucose monomers from glycogen in the form of glucose 1-phosphate molecules. This pathway amplifies the hormonal signal: One receptor protein can activate approximately 100 molecules of G protein, and each enzyme in the pathway, once activated, can act on many molecules of its substrate, the next molecule in the cascade. The number of activated molecules given for each step is approximate.



VISUAL SKILLS ► In the figure, how many glucose 1-phosphate molecules are released in response to one signaling molecule? Calculate the factor by which the response is amplified in going from each step to the next.



Animation: Cytoplasmic Response: Glycogen Breakdown

of activated products can be much greater than in the preceding step. For example, in the epinephrine-triggered pathway in Figure 9.16, each adenylyl cyclase molecule catalyzes the formation of 100 or so cAMP molecules, each molecule of protein kinase A phosphorylates about 10 molecules of the next kinase in the pathway, and so on. The amplification effect stems from the fact that these proteins persist in their active form long enough to process multiple molecules of substrate before they become inactive again. As a result of the signal's amplification, a small number of epinephrine molecules binding to receptors on the surface of a liver cell or muscle cell can lead to the release of hundreds of millions of glucose molecules from glycogen.

The Specificity of Cell Signaling and Coordination of the Response

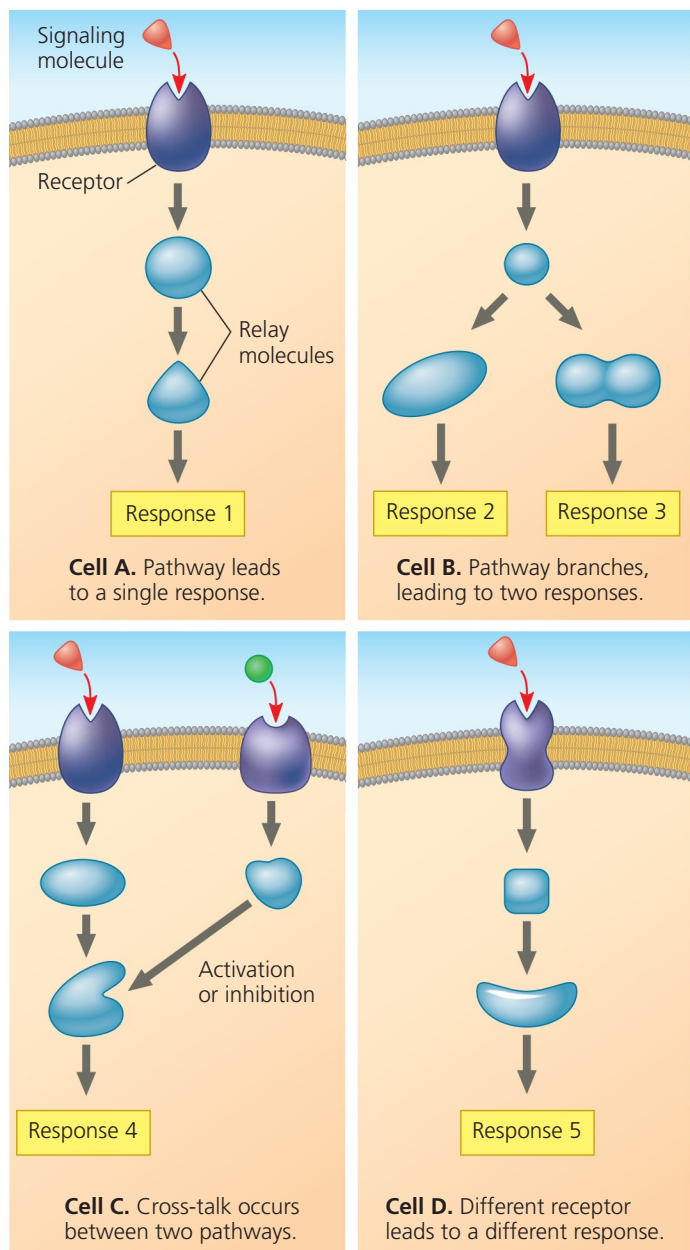
Consider two different cells in your body—a liver cell and a heart muscle cell, for example. Both are in contact with your bloodstream and are therefore constantly exposed to many different hormone molecules, as well as to local regulators secreted by nearby cells. Yet the liver cell responds to some signals but ignores others, and the same is true for the heart cell. And some kinds of signals trigger responses in both cells—but different responses. For instance, epinephrine stimulates the liver cell to break down glycogen, but the main response of the heart cell to epinephrine is contraction, leading to a more rapid heartbeat. How do we account for this difference?

The explanation for the specificity exhibited in cellular responses to signals is the same as the basic explanation for virtually all differences between cells: Because different kinds of cells turn on different sets of genes, *different kinds of cells have different collections of proteins*. The response of a cell to a signal depends on its particular collection of signal receptor proteins, relay proteins, and proteins needed to carry out the response. A liver cell, for example, is poised to respond appropriately to epinephrine by having the proteins listed in Figure 9.16 as well as those needed to manufacture glycogen.

Thus, two cells that respond differently to the same signal differ in one or more proteins that respond to the signal. Notice in **Figure 9.17** that different pathways may have some molecules in common. For example, cells A, B, and C all use the same receptor protein for the red signaling molecule; differences in other proteins account for their differing responses. In cell D, a different receptor protein is used for the same signaling molecule, leading to yet another response. In cell B, a pathway triggered by one signal diverges to produce two responses; such branched pathways often involve receptor tyrosine kinases (which can activate multiple relay proteins) or second messengers (which can regulate numerous proteins). In cell C, two pathways triggered by separate signals converge to modulate a single response. Branching of pathways and “cross-talk” (interaction) between pathways are

important in regulating and coordinating a cell's responses to information coming in from different sources in the body. (You'll learn more about this coordination in Concept 9.5.) Moreover, the use of some of the same proteins in more than one pathway allows the cell to economize on the number of different proteins it must make.

▼ **Figure 9.17 The specificity of cell signaling.** The particular proteins a cell possesses determine what signaling molecules it responds to and the nature of the response. The four cells in these diagrams respond to the same signaling molecule (red) in different ways because each has a different set of proteins (purple and teal). Note, however, that the same kinds of molecules can participate in more than one pathway.



VISUAL SKILLS ► Study the signaling pathway shown in Figure 9.14, and explain how the situation pictured for cell B in Figure 9.17 could apply to that pathway.



Instructors: The Scientific Skills Exercise “Using Experiments to Test a Model” can be assigned in MasteringBiology. It explores the cellular response of a yeast cell to the signal initiated by a mating factor from a cell of the opposite mating type.

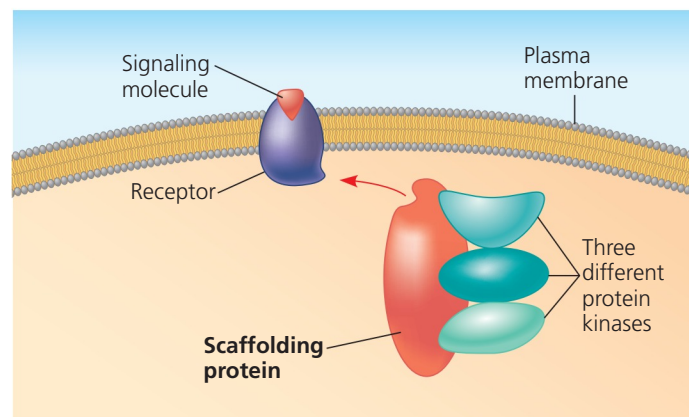
Signaling Efficiency: Scaffolding Proteins and Signaling Complexes

The illustrations of signaling pathways in Figure 9.17 (as well as diagrams of other pathways in this chapter) are greatly simplified. The diagrams show only a few relay molecules and, for clarity's sake, display these molecules spread out in the cytosol. If this were true in the cell, signaling pathways would operate very inefficiently because most relay molecules are proteins, and proteins are too large to diffuse quickly through the viscous cytosol. How does a given protein kinase, for instance, find its protein substrate?

In many cases, the efficiency of signal transduction is apparently increased by the presence of **scaffolding proteins**, large relay proteins to which several other relay proteins are simultaneously attached (**Figure 9.18**). Researchers have found scaffolding proteins in brain cells that *permanently* hold together networks of signaling pathway proteins at synapses. This hardwiring enhances the speed and accuracy of signal transfer between cells because the rate of protein-protein interaction is not limited by diffusion. Furthermore, in some cases the scaffolding proteins themselves may directly activate relay proteins.

The importance of the relay proteins that serve as points of branching or intersection in signaling pathways is highlighted by the problems arising when these proteins are defective or missing. For instance, in an inherited disorder called Wiskott-Aldrich syndrome (WAS), the absence of

▼ **Figure 9.18 A scaffolding protein.** The scaffolding protein shown here simultaneously binds to a specific activated membrane receptor and three different protein kinases. This physical arrangement facilitates signal transduction by these molecules.



a single relay protein leads to such diverse effects as abnormal bleeding, eczema, and a predisposition to infections and leukemia. These symptoms are thought to arise primarily from the absence of the protein in cells of the immune system. By studying normal cells, scientists found that the WAS protein is located just beneath the immune cell surface. The protein interacts both with microfilaments of the cytoskeleton and with several different components of signaling pathways that relay information from the cell surface, including pathways regulating immune cell proliferation. This multifunctional relay protein is thus both a branch point and an important intersection point in a complex signal transduction network that controls immune cell behavior. When the WAS protein is absent, the cytoskeleton is not properly organized and signaling pathways are disrupted, leading to the WAS symptoms.

Termination of the Signal

In the interest of keeping Figure 9.17 simple, we did not show the *inactivation* mechanisms that are an essential aspect of any cell-signaling pathway. For a cell of a multicellular organism to remain capable of responding to incoming signals, each molecular change in its signaling pathways must last only a short time. As we saw in the cholera example, if a signaling pathway component becomes locked into one state, whether active or inactive, consequences for the organism can be serious.

The ability of a cell to receive new signals depends on reversibility of the changes produced by prior signals. The binding of signaling molecules to receptors is reversible. As the external concentration of signaling molecules falls, fewer receptors are bound at any given moment, and the unbound receptors revert to their inactive form. The cellular response occurs only when the concentration of receptors with bound signaling molecules is above a certain threshold. When the number of active receptors falls below that threshold, the cellular response ceases. Then, by a variety of means, the relay molecules return to their inactive forms: The GTPase activity intrinsic to a G protein hydrolyzes its bound GTP; the enzyme phosphodiesterase converts cAMP to AMP; protein phosphatases inactivate phosphorylated kinases and other proteins; and so forth. As a result, the cell is soon ready to respond to a fresh signal.



BioFlix Animation: Mechanism of Hormone Action: Second Messenger cAMP

In this section, we explored the complexity of signaling initiation and termination in a single pathway, and we saw the potential for pathways to intersect with each other. In the next section, we'll consider one especially important network of interacting pathways in the cell.

CONCEPT CHECK 9.4

1. How can a target cell's response to a single hormone molecule result in a response that affects a million other molecules?
2. **WHAT IF? >** If two cells have different scaffolding proteins, explain how they might behave differently in response to the same signaling molecule.
3. **WHAT IF? >** Some human diseases are associated with malfunctioning protein phosphatases. How would such proteins affect signaling pathways? (Review the discussion of protein phosphatases in Concept 9.3, and see Figure 9.10.)

For suggested answers, see Appendix A.

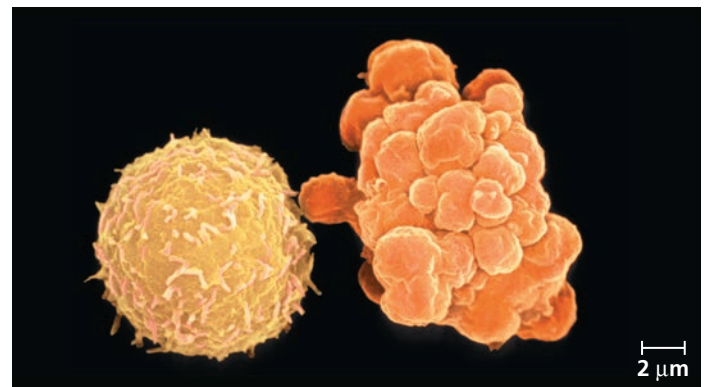
CONCEPT 9.5

Apoptosis integrates multiple cell-signaling pathways

When signaling pathways were first discovered, they were thought to be linear, independent pathways. Our understanding of cellular communication has benefited from the realization that signaling pathway components interact with each other in various ways. For a cell to carry out the appropriate response, cellular proteins often must integrate multiple signals. Let's consider an important cellular process—cellular suicide—as an example.

Cells that are infected, are damaged, or have reached the end of their functional life span often undergo “programmed cell death” (**Figure 9.19**). The best-understood type of this controlled cell suicide is **apoptosis** (from the Greek, meaning “falling off,” and used in a classic Greek poem to refer to leaves falling from a tree). During this process, cellular agents chop up

✓ Figure 9.19 Apoptosis of a human white blood cell. On the left is a normal white blood cell, while on the right is a white blood cell undergoing apoptosis. The apoptotic cell is shrinking and forming lobes (“blebs”), which eventually are shed as membrane-bounded cell fragments (colorized SEMs).



Video: Phosphate-Induced Apoptosis

the DNA and fragment the organelles and other cytoplasmic components. The cell shrinks and becomes lobed (a change called “blebbing”), and the cell’s parts are packaged up in vesicles that are engulfed and digested by specialized scavenger cells, leaving no trace. Apoptosis protects neighboring cells from damage that they would otherwise suffer if a dying cell merely leaked out all its contents, including its many digestive enzymes.

The signal that triggers apoptosis can come from either outside or inside the cell. Outside the cell, signaling molecules released from other cells can initiate a signal transduction pathway that activates the genes and proteins responsible for carrying out cell death. Within a cell whose DNA has been irretrievably damaged, a series of protein-protein interactions can pass along a signal that similarly triggers cell death. Considering some examples of apoptosis can help us to see how signaling pathways are integrated in cells.

Apoptosis in the Soil Worm *Caenorhabditis elegans*

The molecular mechanisms of apoptosis were worked out by researchers studying embryonic development of a small soil worm, a nematode called *Caenorhabditis elegans*. Because the adult worm has only about 1,000 cells, the researchers were able to work out the entire ancestry of each cell. The timely suicide of cells occurs exactly 131 times during normal development of *C. elegans*, at precisely the same points in the cell lineage of each worm. In worms and other species, apoptosis is triggered by signals that activate a cascade of “suicide” proteins in the cells destined to die.

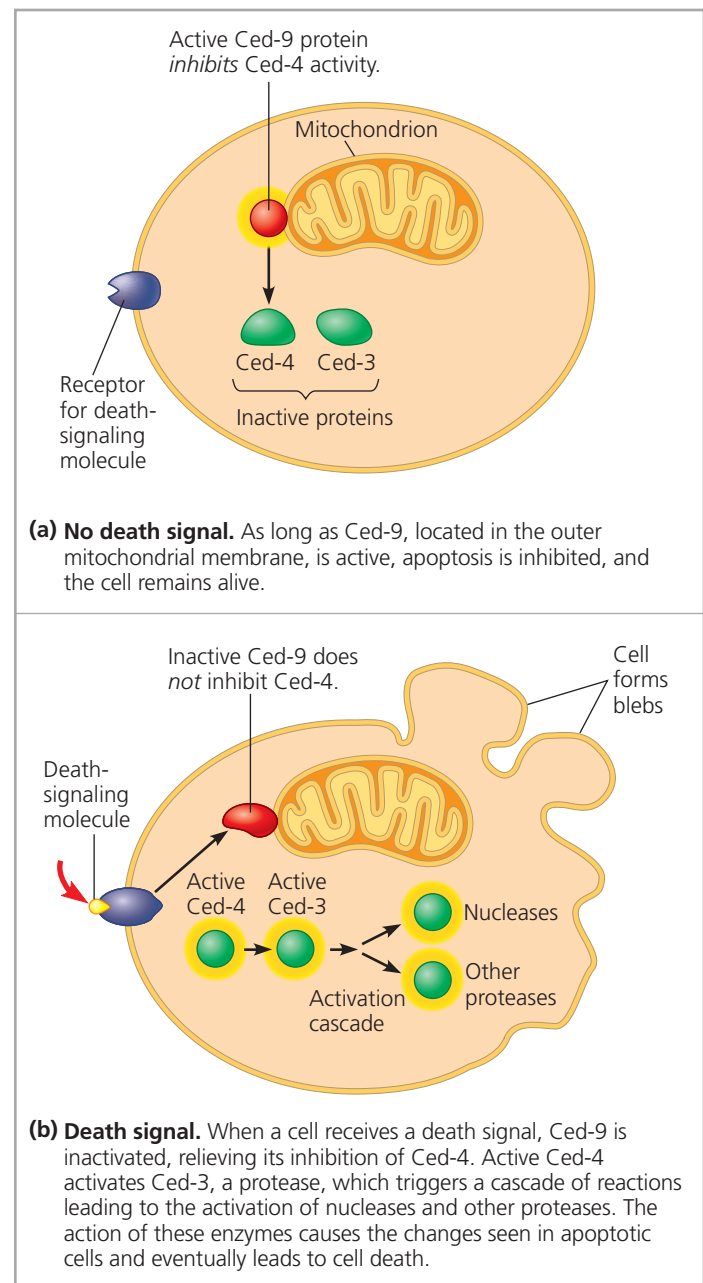
Genetic research on *C. elegans* initially revealed two key apoptosis genes, called *ced-3* and *ced-4* (*ced* stands for “cell death”), which encode proteins essential for apoptosis. The proteins are called Ced-3 and Ced-4, respectively. These and most other proteins involved in apoptosis are continually present in cells, but in inactive form; thus, regulation in this case occurs at the level of protein activity rather than through gene activity and protein synthesis. In *C. elegans*, a protein in the outer mitochondrial membrane, called Ced-9 (the product of the *ced-9* gene), serves as a master regulator of apoptosis, acting as a brake in the absence of a signal promoting apoptosis (Figure 9.20). When a death signal is received by the cell, signal transduction involves a change in Ced-9 that disables the brake, and the apoptotic pathway activates proteases and nucleases, enzymes that cut up the proteins and DNA of the cell. The main proteases of apoptosis are called *caspases*; in the nematode, the chief caspase is the Ced-3 protein.

Apoptotic Pathways and the Signals That Trigger Them

In humans and other mammals, several different pathways, involving about 15 different caspases, can carry out

Figure 9.20 Molecular basis of apoptosis in *C. elegans*.

Three proteins, Ced-3, Ced-4, and Ced-9, are critical to apoptosis and its regulation in the nematode. Apoptosis is more complicated in mammals but involves proteins similar to those in *C. elegans*.



apoptosis. The pathway that is used depends on the type of cell and on the particular signal that initiates apoptosis. One major pathway involves certain mitochondrial proteins that are triggered to form molecular pores in the mitochondrial outer membrane, causing it to leak and release other proteins that promote apoptosis. Perhaps surprisingly, these latter include cytochrome *c*, which functions in mitochondrial ATP synthesis in healthy cells (see Figure 10.15) but acts as a cell death factor when released from mitochondria. The

process of mitochondrial apoptosis in mammals uses proteins similar to the nematode proteins Ced-3, Ced-4, and Ced-9. These can be thought of as relay proteins capable of transducing the apoptotic signal.



Animation: Apoptosis

At key gateways into the apoptotic program, relay proteins integrate signals from several different sources and can send a cell down an apoptotic pathway. Often, the signal originates outside the cell, like the death-signaling molecule depicted in Figure 9.20b, which presumably was released by a neighboring cell. When a death-signaling ligand occupies a cell-surface receptor, this binding leads to activation of caspases and other enzymes that carry out apoptosis, without involving the mitochondrial pathway. This process of signal reception, transduction, and response is similar to what we have discussed throughout this chapter. In a twist on the classic scenario, two other types of alarm signals that can lead to apoptosis originate from *inside* the cell rather than from a cell-surface receptor. One signal comes from the nucleus, generated when the DNA has suffered irreparable damage, and a second comes from the endoplasmic reticulum when excessive protein misfolding occurs. Mammalian cells make life-or-death “decisions” by somehow integrating the death signals and life signals they receive from these external and internal sources.

A built-in cell suicide mechanism is essential to development and maintenance in all animals. The similarities between apoptosis genes in nematodes and those in mammals, as well as the observation that apoptosis occurs in multicellular fungi and even in single-celled yeasts, indicate that the basic mechanism evolved early in the evolution of eukaryotes. In vertebrates, apoptosis is essential for normal development of the nervous system, for normal operation

of the immune system, and for normal morphogenesis of hands and feet in humans and paws in other mammals (**Figure 9.21**). The level of apoptosis between the developing digits is lower in the webbed feet of ducks and other water birds than in the nonwebbed feet of land birds, such as chickens. In the case of humans, the failure of appropriate apoptosis can result in webbed fingers and toes.

Significant evidence points to the involvement of apoptosis in certain degenerative diseases of the nervous system, such as Parkinson’s disease and Alzheimer’s disease. In Alzheimer’s disease, an accumulation of aggregated proteins in neuronal cells activates an enzyme that triggers apoptosis, resulting in the loss of brain function seen in these patients. Furthermore, cancer can result from a failure of cell suicide; some cases of human melanoma, for example, have been linked to faulty forms of the human version of the *C. elegans* Ced-4 protein. It is not surprising, therefore, that the signaling pathways feeding into apoptosis are quite elaborate. After all, the life-or-death question is the most fundamental one imaginable for a cell.

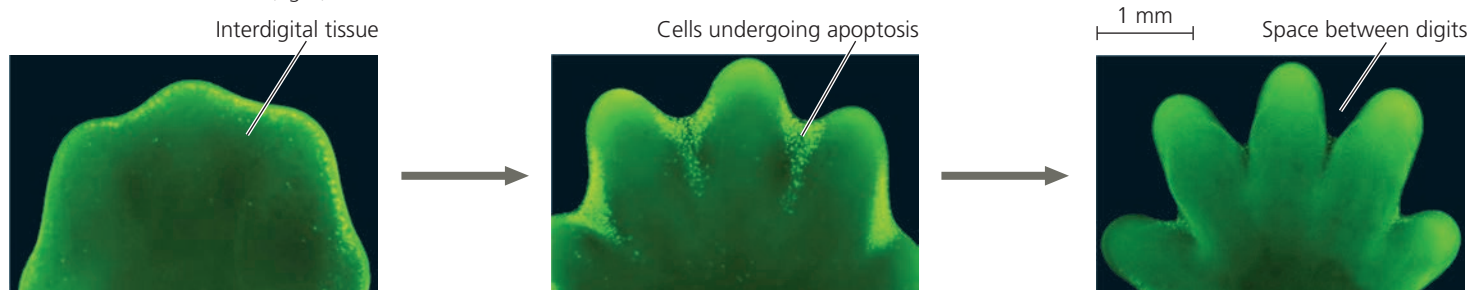
This chapter has introduced you to many of the general mechanisms of cell communication, such as ligand binding, protein-protein interactions and shape changes, cascades of interactions, and protein phosphorylation. Throughout your study of biology, you will encounter numerous examples of cell signaling.

CONCEPT CHECK 9.5

1. Give an example of apoptosis during embryonic development, and explain its function in the developing embryo.
2. **WHAT IF? >** If apoptosis occurred when it should not, what types of protein defects might be the cause? What types could result in apoptosis not occurring when it should?

For suggested answers, see Appendix A.

▼ **Figure 9.21 Effect of apoptosis during paw development in the mouse.** In mice, humans, other mammals, and land birds, the embryonic region that develops into feet or hands initially has a solid, platelike structure. Apoptosis eliminates the cells in the interdigital regions, thus forming the digits. The embryonic mouse paws shown in these fluorescence light micrographs are stained so that cells undergoing apoptosis appear a bright yellowish green. Apoptosis of cells begins at the margin of each interdigital region (left), peaks as the tissue in these regions is reduced (middle), and is no longer visible when the interdigital tissue has been eliminated (right).





SUMMARY OF KEY CONCEPTS

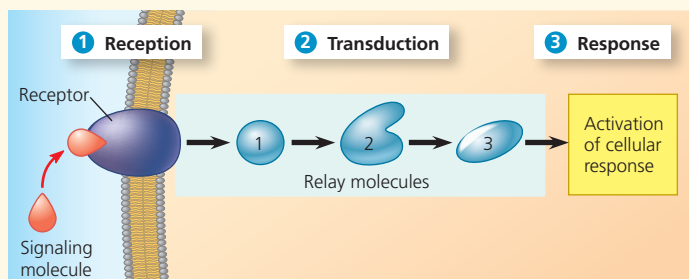
CONCEPT 9.1

External signals are converted to responses within the cell (pp. 215–219)

- **Signal transduction pathways** are crucial for many processes. Signaling during yeast cell mating has much in common with processes in multicellular organisms, suggesting an early evolutionary origin of signaling mechanisms. Bacterial cells can sense the local density of bacterial cells (quorum sensing).
- Local signaling by animal cells involves direct contact or the secretion of local regulators. For long-distance signaling, animal and plant cells use **hormones**; animals also pass signals electrically.
- Like epinephrine, other hormones that bind to membrane receptors trigger a three-stage cell-signaling pathway:



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax



- ? What determines whether a cell responds to a hormone such as epinephrine? What determines how a cell responds to such a hormone?

CONCEPT 9.2

Reception: A signaling molecule binds to a receptor protein, causing it to change shape (pp. 219–223)

- The binding between signaling molecule (**ligand**) and receptor is highly specific. A specific shape change in a receptor is often the initial transduction of the signal.
- There are three major types of cell-surface transmembrane receptors: (1) **G protein-coupled receptors (GPCRs)** work with cytoplasmic **G proteins**. Ligand binding activates the receptor, which then activates a specific G protein, which activates yet another protein, thus propagating the signal. (2) **Receptor tyrosine kinases (RTKs)** react to the binding of signaling molecules by forming dimers and then adding phosphate groups to tyrosines on the cytoplasmic part of the other monomer making up the dimer. Relay proteins in the cell can then be activated by binding to different phosphorylated tyrosines, allowing this receptor to trigger several pathways at once. (3) **Ligand-gated ion channels** open or close in response to binding by specific signaling molecules, regulating the flow of specific ions across the membrane.
- The activity of all three types of receptors is crucial; abnormal GPCRs and RTKs are associated with many human diseases.
- Intracellular receptors are cytoplasmic or nuclear proteins. Signaling molecules that are hydrophobic or small enough to cross the plasma membrane bind to these receptors inside the cell.

- ? How are the structures of a GPCR and an RTK similar? How does initiation of signal transduction differ for these two types of receptors?

CONCEPT 9.3

Transduction: Cascades of molecular interactions relay signals from receptors to target molecules in the cell (pp. 223–227)

- At each step in a signal transduction pathway, the signal is transduced into a different form, which commonly involves a shape change in a protein. Many signal transduction pathways include **phosphorylation cascades**, in which a series of **protein kinases** each add a phosphate group to the next one in line, activating it. Enzymes called **protein phosphatases** remove the phosphate groups. The balance between phosphorylation and dephosphorylation regulates the activity of proteins involved in the sequential steps of a signal transduction pathway.
- **Second messengers**, such as the small molecule **cyclic AMP (cAMP)** and the ion Ca^{2+} , diffuse readily through the cytosol and thus help broadcast signals quickly. Many G proteins activate **adenylyl cyclase**, which makes cAMP from ATP. Cells use Ca^{2+} as a second messenger in both GPCR and RTK pathways. The tyrosine kinase pathways can also involve two other second messengers, **diacylglycerol (DAG)** and **inositol trisphosphate (IP_3)**. IP_3 can trigger a subsequent increase in Ca^{2+} levels.

- ? What is the difference between a protein kinase and a second messenger? Can both operate in the same signal transduction pathway?

CONCEPT 9.4

Response: Cell signaling leads to regulation of transcription or cytoplasmic activities (pp. 228–231)

- Some pathways lead to a nuclear response: Specific genes are turned on or off by activated transcription factors. In others, the response involves cytoplasmic regulation.
- Cellular responses are not simply on or off; they are regulated at many steps. Each protein in a signaling pathway amplifies the signal by activating multiple copies of the next component; for long pathways, the total amplification may be over a millionfold. The combination of proteins in a cell confers specificity in the signals it detects and the responses it carries out. **Scaffolding proteins** increase signaling efficiency. Pathway branching further helps the cell coordinate signals and responses. Signal response can be terminated quickly because ligand binding is reversible.

- ? What mechanisms in the cell terminate its response to a signal and maintain its ability to respond to new signals?

CONCEPT 9.5

Apoptosis integrates multiple cell-signaling pathways (pp. 231–233)

- **Apoptosis** is a type of programmed cell death in which cell components are disposed of in an orderly fashion. Studies of the soil worm *Caenorhabditis elegans* clarified molecular details of the relevant signaling pathways. A death signal leads to activation of caspases and nucleases, the main enzymes involved in apoptosis.
- Several apoptotic signaling pathways exist in the cells of humans and other mammals, triggered in different ways. Signals eliciting apoptosis can originate from outside or inside the cell.

- ? What is an explanation for the similarities between genes in yeasts, nematodes, and mammals that control apoptosis?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. **DRAW IT** Draw the following apoptotic pathway, which operates in human immune cells. A death signal is received when a molecule called Fas binds its cell-surface receptor. The binding of many Fas molecules to receptors causes receptor clustering. The intracellular regions of the receptors, when together, bind proteins called adaptor proteins. These in turn bind to inactive molecules of caspase-8, which become activated and then activate caspase-3. Once activated, caspase-3 initiates apoptosis.
9. **EVOLUTION CONNECTION** Identify the evolutionary mechanisms that might account for the origin and persistence of cell-to-cell signaling systems in prokaryotes.
10. **SCIENTIFIC INQUIRY** Epinephrine initiates a signal transduction pathway that produces cyclic AMP (cAMP) and leads to the breakdown of glycogen to glucose, a major energy source for cells. But glycogen breakdown is only part of the fight-or-flight response that epinephrine brings about; the overall effect on the body includes an increase in heart rate and alertness, as well as a burst of energy. Given that caffeine blocks the activity of cAMP phosphodiesterase, propose a mechanism by which caffeine ingestion leads to heightened alertness and sleeplessness.
11. **SCIENCE, TECHNOLOGY, AND SOCIETY** The aging process is thought to be initiated at the cellular level. Among the changes that can occur after a certain number of cell divisions is the loss of a cell's ability to respond to growth factors and other signals. Much research into aging is aimed at understanding such losses, with the ultimate goal of extending the human life span. Not everyone, however, agrees that this is a desirable goal. If life expectancy were greatly increased, discuss what might be the social and ecological consequences.
12. **WRITE ABOUT A THEME: ORGANIZATION** The properties of life emerge at the biological level of the cell. The highly regulated process of apoptosis is not simply the destruction of a



cell; it is also an emergent property. Write a short essay (about 100–150 words) that briefly explains the role of apoptosis in the development and proper functioning of an animal, and describe how this form of programmed cell death is a process that emerges from the orderly integration of signaling pathways.

13. SYNTHESIZE YOUR KNOWLEDGE



There are five basic tastes—sour, salty, sweet, bitter, and “umami.” Salt is detected when the concentration of salt outside of a taste bud cell is higher than that inside of it, and ion channels allow the passive leakage of Na^+ into the cell. The resulting change in membrane potential (see Concept 8.4) sends the “salty” signal to the brain. Umami is a savory taste generated by glutamate (glutamic acid, found in monosodium glutamate, or MSG), which is used as a flavor enhancer in foods such as taco-flavored tortilla chips. The glutamate receptor is a GPCR, which, when bound, initiates a signaling pathway that ends with a cellular response, perceived by you as “taste.” If you eat a regular potato chip and then rinse your mouth, you will no longer taste salt. But if you eat a flavored tortilla chip and then rinse, the taste persists. (Try it!) Propose a possible explanation for this difference.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Cell Respiration

10



▲ **Figure 10.1** How does food, like the sand eels captured by this puffin, power the work of life?

KEY CONCEPTS

- 10.1** Catabolic pathways yield energy by oxidizing organic fuels
- 10.2** Glycolysis harvests chemical energy by oxidizing glucose to pyruvate
- 10.3** After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules
- 10.4** During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis
- 10.5** Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen
- 10.6** Glycolysis and the citric acid cycle connect to many other metabolic pathways

Life Is Work

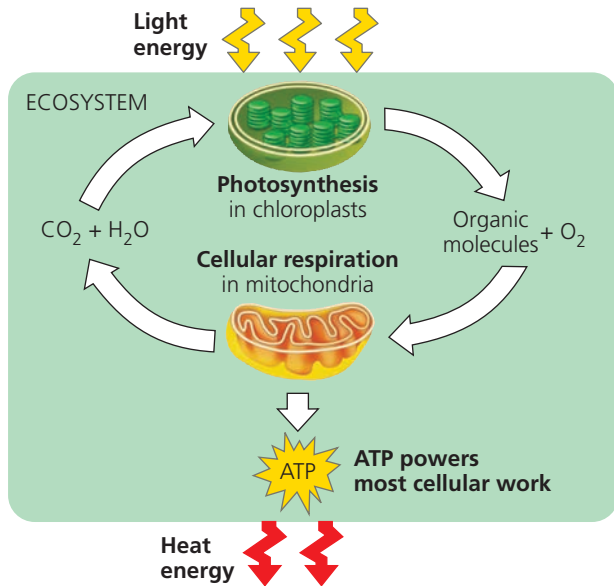
Living cells require transfusions of energy from outside sources to perform their many tasks—for example, assembling polymers, pumping substances across membranes, moving, and reproducing. The puffin in **Figure 10.1** obtains energy for its cells by feeding upon sand eels and other aquatic organisms; many other animals obtain energy by feeding on photosynthetic organisms such as plants and algae.

The energy stored in the organic molecules of food ultimately comes from the sun. Energy flows into an ecosystem as sunlight and exits as heat; in contrast, the chemical elements essential to life are recycled (**Figure 10.2**). Photosynthesis generates oxygen, as well as organic molecules used by the mitochondria of eukaryotes as fuel for cellular respiration. Respiration breaks this fuel down, using oxygen and generating ATP. The waste products of this type of respiration, carbon dioxide and water, are the raw materials for photosynthesis.

In this chapter, we consider how cells harvest the chemical energy stored in organic molecules and use it to generate ATP, the molecule that drives most cellular work. After presenting some basic information about respiration, we'll focus on three key pathways of respiration: glycolysis, pyruvate oxidation and the citric acid cycle, and oxidative phosphorylation. We'll also consider fermentation, a somewhat simpler pathway coupled to glycolysis that has deep evolutionary roots.



▼ **Figure 10.2 Energy flow and chemical recycling in ecosystems.** Energy flows into an ecosystem as sunlight and ultimately leaves as heat, while the chemical elements essential to life are recycled.



 Animation: Energy Flow and Chemical Recycling

CONCEPT 10.1

Catabolic pathways yield energy by oxidizing organic fuels

Metabolic pathways that release stored energy by breaking down complex molecules are called catabolic pathways (see Concept 6.1). Transfer of electrons from fuel molecules (like glucose) to other molecules plays a major role in these pathways. In this section, we consider these processes, which are central to cellular respiration.

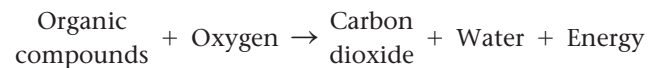
Catabolic Pathways and Production of ATP

Organic compounds possess potential energy as a result of the arrangement of electrons in the bonds between their atoms. Compounds that can participate in exergonic reactions can act as fuels. Through the activity of enzymes, a cell systematically degrades complex organic molecules that are rich in potential energy to simpler waste products that have less energy. Some of the energy taken out of chemical storage can be used to do work; the rest is dissipated as heat.

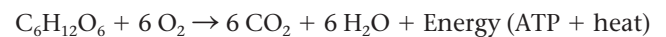
One catabolic process, **fermentation**, is a partial degradation of sugars or other organic fuel that occurs without the use of oxygen. However, the most efficient catabolic pathway is **aerobic respiration**, in which oxygen is consumed as a reactant along with the organic fuel (*aerobic* is from the Greek *aer*, air, and *bios*, life). The cells of most eukaryotic and many prokaryotic organisms can carry out aerobic respiration. Some prokaryotes use substances other than oxygen as reactants in a similar process that harvests chemical energy without oxygen;

this process is called *anaerobic respiration* (the prefix *an-* means “without”). Technically, the term **cellular respiration** includes both aerobic and anaerobic processes. However, it originated as a synonym for aerobic respiration because of the relationship of that process to organismal respiration, in which an animal breathes in oxygen. Thus, *cellular respiration* is often used to refer to the aerobic process, a practice we follow in most of this chapter.

Although very different in mechanism, aerobic respiration is in principle similar to the combustion of gasoline in an automobile engine after oxygen is mixed with the fuel (hydrocarbons). Food provides the fuel for respiration, and the exhaust is carbon dioxide and water. The overall process can be summarized as follows:



Carbohydrates, fats, and protein molecules from food can all be processed and consumed as fuel, as we will discuss later in the chapter. In animal diets, a major source of carbohydrates is starch, a storage polysaccharide that can be broken down into glucose (C₆H₁₂O₆) subunits. Here, we will learn the steps of cellular respiration by tracking the degradation of the sugar glucose:



This breakdown of glucose is exergonic, having a free-energy change of -686 kcal ($2,870 \text{ kJ}$) per mole of glucose decomposed ($\Delta G = -686 \text{ kcal/mol}$). Recall that a negative ΔG ($\Delta G < 0$) indicates that the products of the chemical process store less energy than the reactants and that the reaction can happen spontaneously—in other words, without an input of energy.

Catabolic pathways do not directly move flagella, pump solutes across membranes, polymerize monomers, or perform other cellular work. Catabolism is linked to work by a chemical drive shaft—ATP (see Concept 6.3). To keep working, the cell must regenerate its supply of ATP from ADP and P_i (see Figure 6.12). To understand how cellular respiration accomplishes this, let's examine the fundamental chemical processes known as oxidation and reduction.

Redox Reactions: Oxidation and Reduction

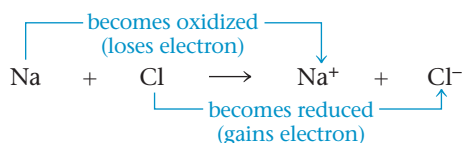
How do the catabolic pathways that decompose glucose and other organic fuels yield energy? The answer is based on the transfer of electrons during the chemical reactions. The relocation of electrons releases energy stored in organic molecules, and this energy ultimately is used to synthesize ATP.

The Principle of Redox

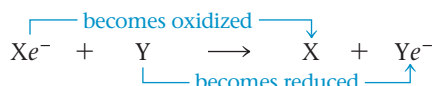
In many chemical reactions, there is a transfer of one or more electrons (e^-) from one reactant to another. These electron transfers are called oxidation-reduction reactions,

or **redox reactions** for short. In a redox reaction, the loss of electrons from one substance is called **oxidation**, and the addition of electrons to another substance is known as **reduction**. (Note that *adding* electrons is called *reduction*; adding negatively charged electrons to an atom *reduces* the amount of positive charge of that atom.)

To take a simple, nonbiological example, consider the reaction between the elements sodium (Na) and chlorine (Cl) that forms table salt:



We could generalize a redox reaction this way:



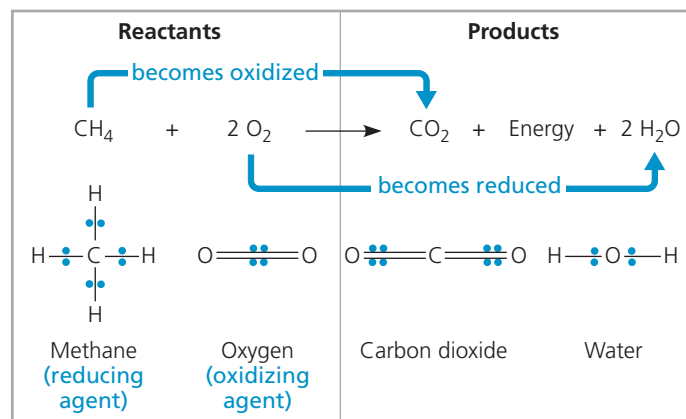
In the generalized reaction, substance Xe^- , the electron donor, is called the **reducing agent**; it reduces Y, which accepts the donated electron. Substance Y, the electron acceptor, is the **oxidizing agent**; it oxidizes Xe^- by removing its electron. Because an electron transfer requires both an electron donor and an acceptor, oxidation and reduction always go hand in hand.

Not all redox reactions involve the complete transfer of electrons from one substance to another; some change the degree of electron sharing in covalent bonds. Methane combustion, shown in **Figure 10.3**, is an example. The covalent electrons in methane are shared nearly equally between the bonded atoms because carbon and hydrogen have about the same affinity for valence electrons; they are about equally electronegative (see Concept 2.3). But when methane reacts with oxygen, forming carbon dioxide, electrons end up shared less equally between the carbon atom and its new covalent partners, the oxygen atoms, which are very electronegative. In effect, the carbon atom has partially “lost” its shared electrons; thus, methane has been oxidized.

Now let’s examine the fate of the reactant O_2 . The two atoms of the oxygen molecule (O_2) share their electrons equally. But when oxygen reacts with the hydrogen from methane, forming water, the electrons of the covalent bonds spend more time near the oxygen (see Figure 10.3). In effect, each oxygen atom has partially “gained” electrons, so the oxygen molecule has been reduced. Because oxygen is so electronegative, it is one of the most powerful of all oxidizing agents.

Energy must be added to pull an electron away from an atom, just as energy is required to push a ball uphill. The more electronegative the atom (the stronger its pull on electrons), the more energy is required to take an electron away from it. An electron loses potential energy when it shifts from a less electronegative atom toward a more electronegative one, just as a ball loses potential energy when it rolls downhill. A redox reaction that moves electrons closer to oxygen,

▼ **Figure 10.3 Methane combustion as an energy-yielding redox reaction.** The reaction releases energy to the surroundings because the electrons lose potential energy when they end up being shared unequally, spending more time near electronegative atoms such as oxygen.

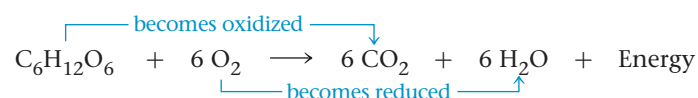


Animation: Redox Reactions

such as the burning (oxidation) of methane, therefore releases chemical energy that can be put to work.

Oxidation of Organic Fuel Molecules During Cellular Respiration

The oxidation of methane by oxygen is the main combustion reaction that occurs at the burner of a gas stove. The combustion of gasoline in an automobile engine is also a redox reaction; the energy released pushes the pistons. But the energy-yielding redox process of greatest interest to biologists is respiration: the oxidation of glucose and other molecules in food. Examine again the summary equation for cellular respiration, but this time think of it as a redox process:



As in the combustion of methane or gasoline, the fuel (glucose) is oxidized and oxygen is reduced. The electrons lose potential energy along the way, and energy is released.

In general, organic molecules that have an abundance of hydrogen are excellent fuels because their bonds are a source of “hilltop” electrons, whose energy may be released as these electrons “fall” down an energy gradient during their transfer to oxygen. The summary equation for respiration indicates that hydrogen is transferred from glucose to oxygen. But the important point, not visible in the summary equation, is that the energy state of the electron changes as hydrogen (with its electron) is transferred to oxygen. In respiration, the oxidation of glucose transfers electrons to a lower energy state, liberating energy that becomes available for ATP synthesis. So, in general, we see fuels with multiple C—H bonds oxidized into products with multiple C—O bonds.

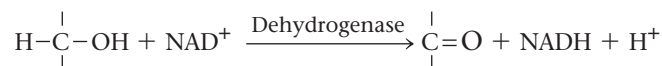
The main energy-yielding foods—carbohydrates and fats—are reservoirs of electrons associated with hydrogen, often in the form of C—H bonds. Only the barrier of activation energy holds back the flood of electrons to a lower energy state (see Figure 6.13). Without this barrier, a food substance like glucose would combine almost instantaneously with O₂. If we supply the activation energy by igniting glucose, it burns in air, releasing 686 kcal (2,870 kJ) of heat per mole of glucose (about 180 g). Body temperature is not high enough to initiate burning, of course. Instead, if you swallow some glucose, enzymes in your cells will lower the barrier of activation energy, allowing the sugar to be oxidized in a series of steps.

Stepwise Energy Harvest via NAD⁺ and the Electron Transport Chain

If energy is released from a fuel all at once, it cannot be harnessed efficiently for constructive work. For example, if a gasoline tank explodes, it cannot drive a car very far. Cellular respiration does not oxidize glucose (or any other organic fuel) in a single explosive step either. Rather, glucose is broken down in a series of steps, each one catalyzed by an enzyme. At key steps, electrons are stripped from the glucose. As is often the case in oxidation reactions, each electron travels with a proton—thus, as a hydrogen atom. The hydrogen atoms are not transferred directly to oxygen, but instead are usually passed first to an electron carrier, a coenzyme called nicotinamide adenine dinucleotide, a derivative of the vitamin niacin. This coenzyme is well suited as an electron carrier because it can cycle easily between its oxidized form, **NAD⁺**, and its reduced form, **NADH**. As an electron acceptor, NAD⁺ functions as an oxidizing agent during respiration.

How does NAD⁺ trap electrons from glucose and the other organic molecules in food? Enzymes called dehydrogenases remove a pair of hydrogen atoms (2 electrons and 2 protons)

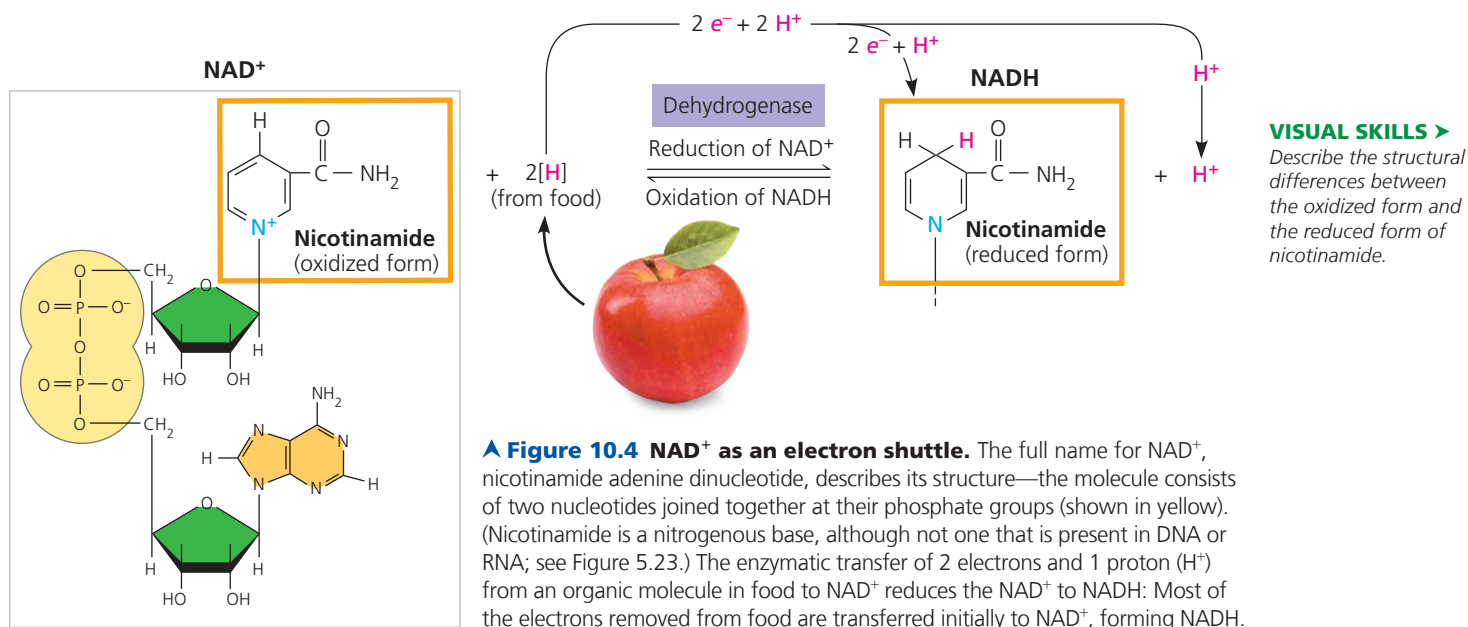
from the substrate (glucose, in the preceding example), thereby oxidizing it. The enzyme delivers the 2 electrons along with 1 proton to its coenzyme, NAD⁺, forming NADH (**Figure 10.4**). The other proton is released as a hydrogen ion (H⁺) into the surrounding solution:



By receiving 2 negatively charged electrons but only 1 positively charged proton, the nicotinamide portion of NAD⁺ has its charge neutralized when NAD⁺ is reduced to NADH. The name NADH shows the hydrogen that has been received in the reaction. NAD⁺ is the most versatile electron acceptor in cellular respiration and functions in several of the redox steps during the breakdown of glucose.

Electrons lose very little of their potential energy when they are transferred from glucose to NAD⁺. Each NADH molecule formed during respiration represents stored energy. This energy can be tapped to make ATP when the electrons complete their “fall” in a series of steps down an energy gradient from NADH to oxygen.

How do electrons that are extracted from glucose and stored as potential energy in NADH finally reach oxygen? It will help to compare the redox chemistry of cellular respiration to a much simpler reaction: the reaction between hydrogen and oxygen to form water (**Figure 10.5a**). Mix H₂ and O₂, provide a spark for activation energy, and the gases combine explosively. In fact, combustion of liquid H₂ and O₂ is harnessed to help power the rocket engines that boost satellites into orbit and launch spacecraft. The explosion represents a release of energy as the electrons of hydrogen “fall” closer to the electronegative oxygen atoms. Cellular respiration also brings hydrogen and oxygen together to form water, but there are two important differences. First, in cellular respiration, the hydrogen that reacts with oxygen



is derived from organic molecules rather than H_2 . Second, instead of occurring in one explosive reaction, respiration uses an electron transport chain to break the fall of electrons to oxygen into several energy-releasing steps (**Figure 10.5b**). An **electron transport chain** consists of a number of molecules, mostly proteins, built into the inner membrane of the mitochondria of eukaryotic cells (and the plasma membrane of respiring prokaryotes). Electrons removed from glucose are shuttled by NADH to the “top,” higher-energy end of the chain. At the “bottom,” lower-energy end, O_2 captures these electrons along with hydrogen nuclei (H^+), forming water. (Anaerobically respiring prokaryotes have an electron acceptor at the end of the chain that is different from O_2 .)

Electron transfer from NADH to oxygen is an exergonic reaction with a free-energy change of -53 kcal/mol (-222 kJ/mol). Instead of this energy being released and wasted in a single explosive step, electrons cascade down the chain from one carrier molecule to the next in a series of redox reactions, losing a small amount of energy with each step until they finally reach oxygen, the terminal electron acceptor, which has a very great affinity for electrons. Each “downhill” carrier is more electronegative than, and thus capable of oxidizing, its “uphill” neighbor, with oxygen at the bottom of the chain. Therefore, the electrons transferred from glucose to NAD^+ , which is thus reduced to NADH, fall down an energy gradient in the electron transport chain to a far more stable location in the electronegative oxygen atom. Put another way, oxygen pulls electrons

down the chain in an energy-yielding tumble analogous to gravity pulling objects downhill.

In summary, during cellular respiration, most electrons travel the following “downhill” route: glucose $\rightarrow$ NADH $\rightarrow$ electron transport chain $\rightarrow$ oxygen. Later in this chapter, you will learn more about how the cell uses the energy released from this exergonic electron fall to regenerate its supply of ATP. For now, having covered the basic redox mechanisms of cellular respiration, let’s look at the entire process by which energy is harvested from organic fuels.

The Stages of Cellular Respiration: A Preview

The harvesting of energy from glucose by cellular respiration is a cumulative function of three metabolic stages. We list them here along with a color-coding scheme we will use throughout the chapter to help you keep track of the big picture:

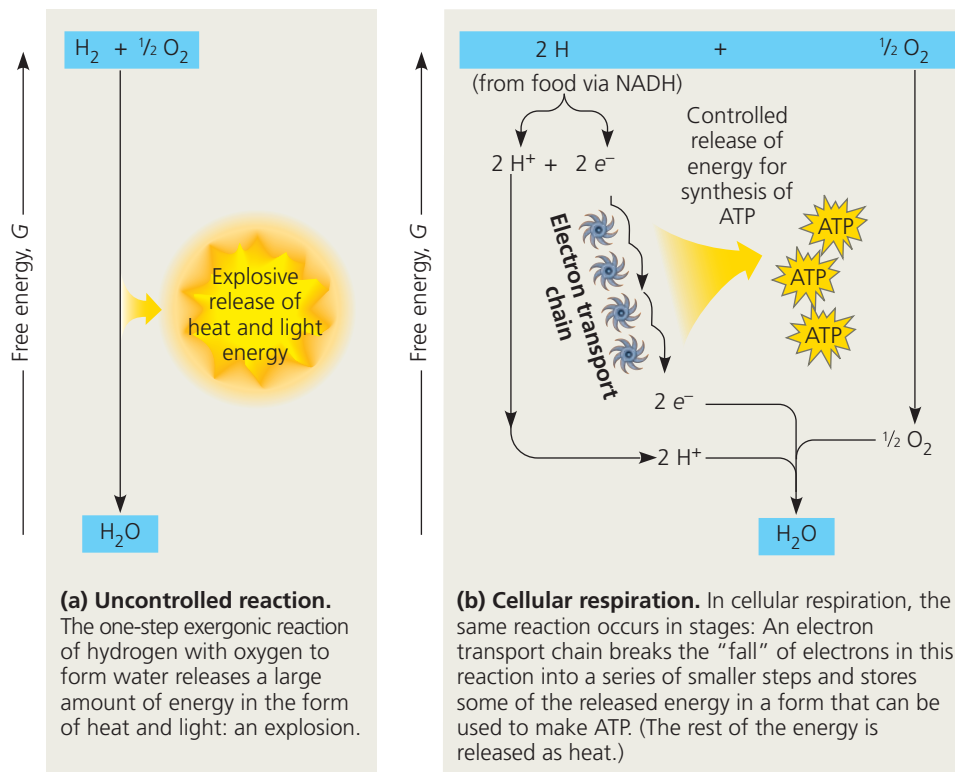
1. **GLYCOLYSIS** (color-coded blue throughout the chapter)
2. **PYRUVATE OXIDATION and the CITRIC ACID CYCLE** (color-coded light orange and dark orange)
3. **OXIDATIVE PHOSPHORYLATION: Electron transport and chemiosmosis** (color-coded purple)

Biochemists usually reserve the term *cellular respiration* for stages 2 and 3 together. In this text, however, we include glycolysis as a part of cellular respiration because most respiring cells deriving energy from glucose use glycolysis to produce the starting material for the citric acid cycle.

As diagrammed in **Figure 10.6**, glycolysis and then pyruvate oxidation and the citric acid cycle are the catabolic pathways that break down glucose and other organic fuels. **Glycolysis**, which occurs in the cytosol, begins the degradation process by breaking glucose into two molecules of a compound called pyruvate. In eukaryotes, pyruvate enters the mitochondrion and is oxidized to a compound called acetyl CoA, which enters the **citric acid cycle**. There, the breakdown of glucose to carbon dioxide is completed. (In prokaryotes, these processes take place in the cytosol.) Thus, the carbon dioxide produced by respiration represents fragments of oxidized organic molecules.

Some of the steps of glycolysis and the citric acid cycle are redox reactions in which dehydrogenases transfer electrons from substrates to NAD^+ or the related electron carrier FAD, forming NADH or FADH_2 . (You’ll learn more about FAD and FADH_2 later.) In the third stage of respiration, the electron transport chain

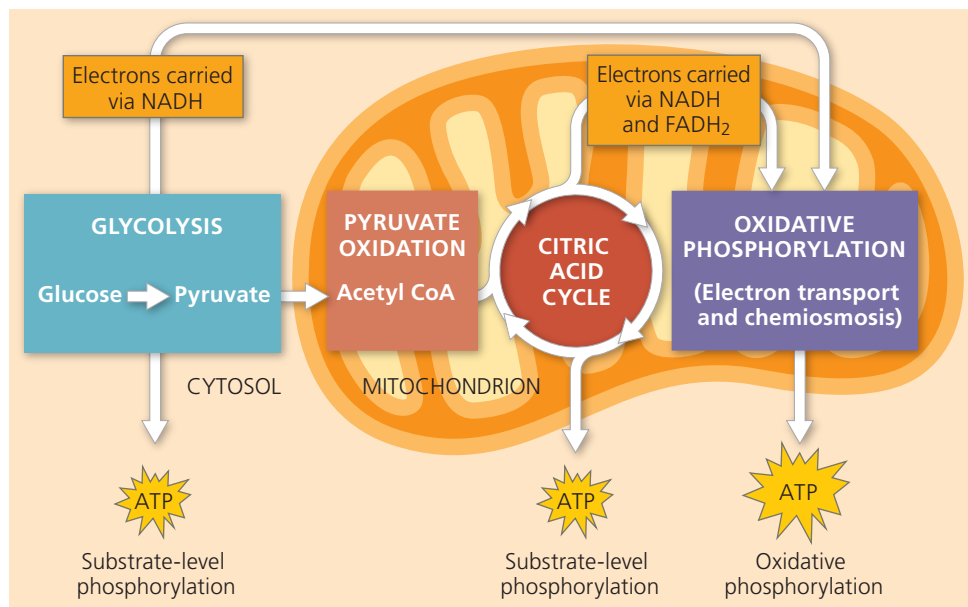
Figure 10.5 An introduction to electron transport chains.



➤ **Figure 10.6 An overview of cellular respiration.** During glycolysis, each glucose molecule is broken down into two molecules of pyruvate. In eukaryotic cells, as shown here, the pyruvate enters the mitochondrion. There it is oxidized to acetyl CoA, which will be further oxidized to CO₂ in the citric acid cycle. The electron carriers NADH and FADH₂ transfer electrons derived from glucose to electron transport chains. During oxidative phosphorylation, electron transport chains convert the chemical energy to a form used for ATP synthesis in the process called chemiosmosis. (During earlier steps of cellular respiration, a few molecules of ATP are synthesized in a process called substrate-level phosphorylation.) To visualize these processes in their cellular context, see Figure 7.32.



Animation: Overview of Cellular Respiration



accepts electrons from NADH or FADH₂ generated during the first two stages and passes these electrons down the chain. At the end of the chain, the electrons are combined with molecular oxygen and hydrogen ions (H⁺), forming water (see Figure 10.5b). The energy released at each step of the chain is stored in a form the mitochondrion (or prokaryotic cell) can use to make ATP from ADP. This mode of ATP synthesis is called **oxidative phosphorylation** because it is powered by the redox reactions of the electron transport chain.

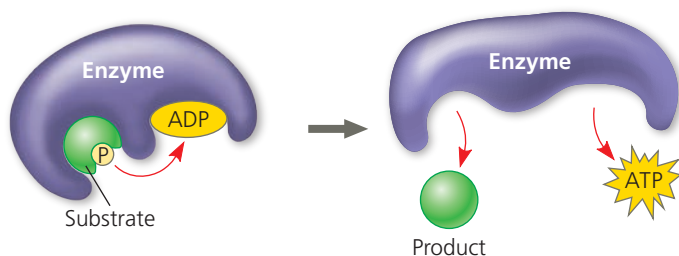
In eukaryotic cells, the inner membrane of the mitochondrion is the site of electron transport and another process called *chemiosmosis*, together making up oxidative phosphorylation. (In prokaryotes, these processes take place in the plasma membrane.) Oxidative phosphorylation accounts for almost 90% of the ATP generated by respiration. A smaller amount of ATP is formed directly in a few reactions of glycolysis and the citric acid cycle by a mechanism called **substrate-level phosphorylation** (Figure 10.7). This mode of ATP synthesis

occurs when an enzyme transfers a phosphate group from a substrate molecule to ADP, rather than adding an inorganic phosphate to ADP as in oxidative phosphorylation. “Substrate molecule” here refers to an organic molecule generated as an intermediate during the catabolism of glucose. You’ll see examples of substrate-level phosphorylation later in the chapter, in both glycolysis and the citric acid cycle.

You can think of the whole process this way: When you withdraw a relatively large sum of money from an ATM, it is not delivered to you in a single bill of larger denomination. Instead, a number of smaller-denomination bills are dispensed that you can spend more easily. This is analogous to ATP production during cellular respiration. For each molecule of glucose degraded to carbon dioxide and water by respiration, the cell makes up to about 32 molecules of ATP, each with 7.3 kcal/mol of free energy. Respiration cashes in the large denomination of energy banked in a single molecule of glucose (686 kcal/mol under standard conditions) for the small change of many molecules of ATP, which is more practical for the cell to spend on its work.

This preview has introduced you to how glycolysis, the citric acid cycle, and oxidative phosphorylation fit into the process of cellular respiration. We are now ready to take a closer look at each of these three stages of respiration.

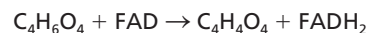
▼ **Figure 10.7 Substrate-level phosphorylation.** Some ATP is made by direct transfer of a phosphate group from an organic substrate to ADP by an enzyme. (For examples in glycolysis, see Figure 10.9, steps 7 and 10.)



MAKE CONNECTIONS ➤ Review Figure 6.9. Do you think the potential energy is higher for the reactants or the products in the reaction shown above? Explain.

CONCEPT CHECK 10.1

1. Compare and contrast aerobic and anaerobic respiration, including the processes involved.
2. **WHAT IF?** ➤ If the following reaction occurred, which compound would be oxidized? Reduced?



For suggested answers, see Appendix A.

CONCEPT 10.2

Glycolysis harvests chemical energy by oxidizing glucose to pyruvate

The word *glycolysis* means “sugar splitting,” and that is exactly what happens during this pathway. Glucose, a six-carbon sugar, is split into two three-carbon sugars. These smaller sugars are then oxidized and their remaining atoms rearranged to form two molecules of pyruvate. (Pyruvate is the ionized form of pyruvic acid.)

As summarized in **Figure 10.8**, glycolysis can be divided into two phases: the energy investment phase and the energy payoff phase. During the energy investment phase, the cell actually spends ATP. This investment is repaid with interest during the energy payoff phase, when ATP is produced by substrate-level phosphorylation and NAD^+ is reduced to NADH by electrons released from the oxidation of glucose. The net energy yield from glycolysis, per glucose molecule, is 2 ATP plus 2 NADH. The ten steps of the glycolytic pathway are shown in **Figure 10.9**.

All of the carbon originally present in glucose is accounted for in the two molecules of pyruvate; no carbon is released as CO_2 during glycolysis. Glycolysis occurs whether or not O_2 is present. However, if O_2 is present, the chemical energy stored in pyruvate and NADH can be extracted by pyruvate oxidation, the citric acid cycle, and oxidative phosphorylation.

Figure 10.8 The energy input and output of glycolysis.

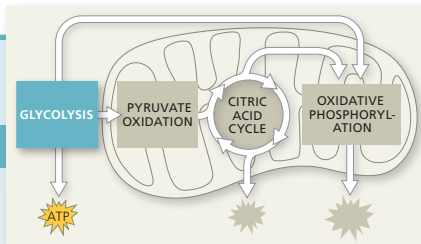
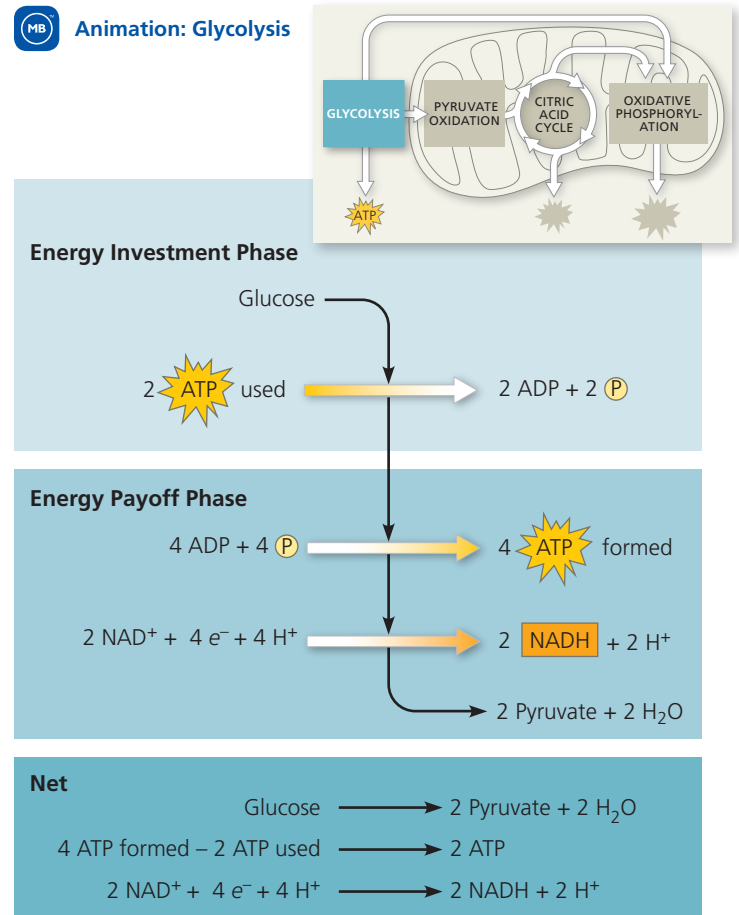
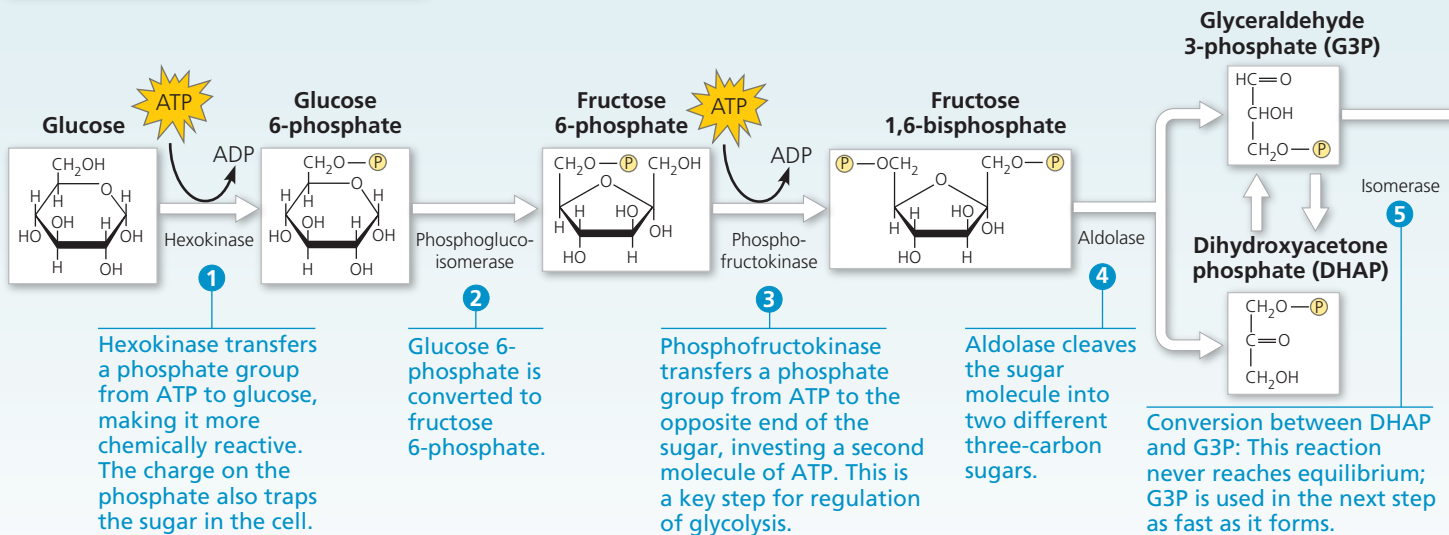


Figure 10.9 A closer look at glycolysis. Note that glycolysis is a source of ATP and NADH.

GLYCOLYSIS: Energy Investment Phase

WHAT IF? > What would happen if you removed the dihydroxyacetone phosphate generated in step 4 as fast as it was produced?



CONCEPT CHECK 10.2

- 1. VISUAL SKILLS** > During the redox reaction in glycolysis (see step 6 in Figure 10.9), which molecule acts as the oxidizing agent? The reducing agent?

For suggested answers, see Appendix A.

CONCEPT 10.3

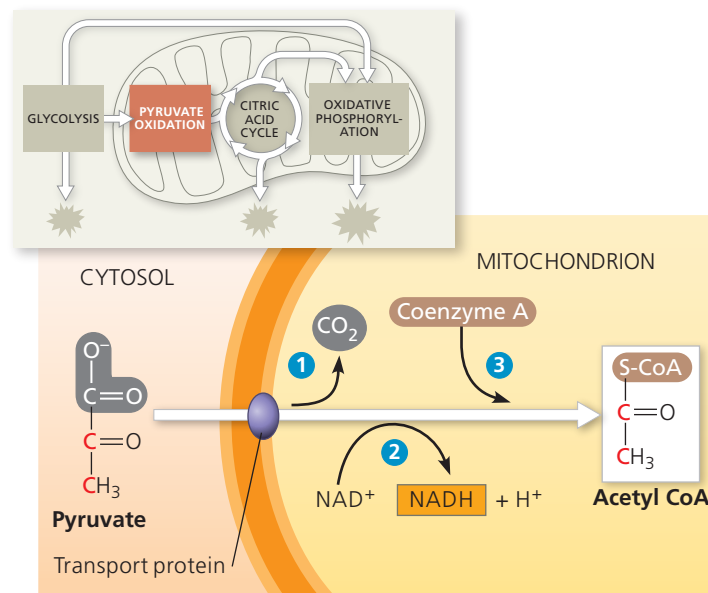
After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules

Glycolysis releases less than a quarter of the chemical energy in glucose that can be harvested by cells; most of the energy remains stockpiled in the two molecules of pyruvate. When O_2 is present, the pyruvate in eukaryotic cells enters a mitochondrion, where the oxidation of glucose is completed. In aerobically respiring prokaryotic cells, this process occurs in the cytosol. (Later in the chapter, we'll discuss what happens to pyruvate when O_2 is unavailable or in a prokaryote that is unable to use O_2 .)

Oxidation of Pyruvate to Acetyl CoA

Upon entering the mitochondrion via active transport, pyruvate is first converted to a compound called acetyl coenzyme A, or **acetyl CoA** (Figure 10.10). This step, linking glycolysis and the citric acid cycle, is carried out by a multienzyme complex that catalyzes three reactions: 1 Pyruvate's carboxyl

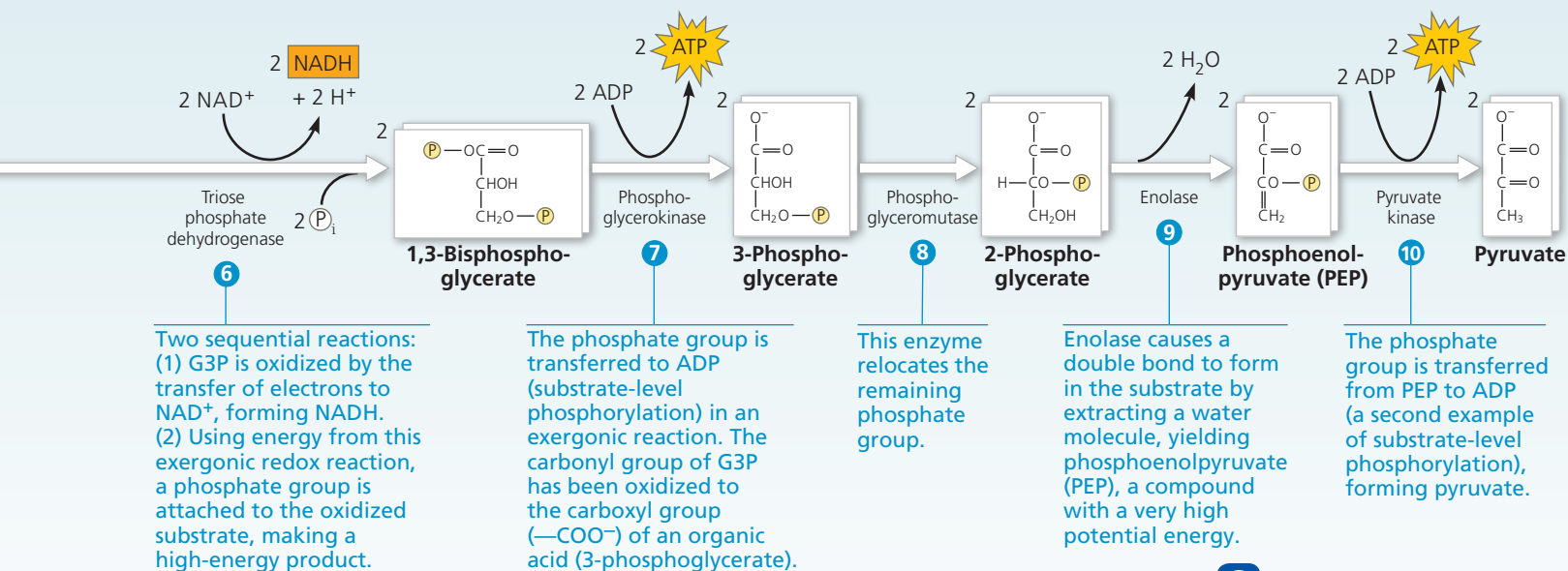
Figure 10.10 Oxidation of pyruvate to acetyl CoA, the step before the citric acid cycle. Pyruvate is a charged molecule, so in eukaryotic cells it must enter the mitochondrion via active transport, with the help of a transport protein. Next, a complex of several enzymes (the pyruvate dehydrogenase complex) catalyzes the three numbered steps, which are described in the text. The acetyl group of acetyl CoA will enter the citric acid cycle. The CO_2 molecule will diffuse out of the cell. By convention, coenzyme A is abbreviated S-CoA when it is attached to a molecule, emphasizing the sulfur atom (S). (The gene encoding the transport protein was finally identified several years ago, after 40 years of research.)



BioFlix® Animation: Acetyl CoA

The energy payoff phase occurs after glucose is split into two three-carbon sugars. Thus, the coefficient 2 precedes all molecules in this phase.

GLYCOLYSIS: Energy Payoff Phase



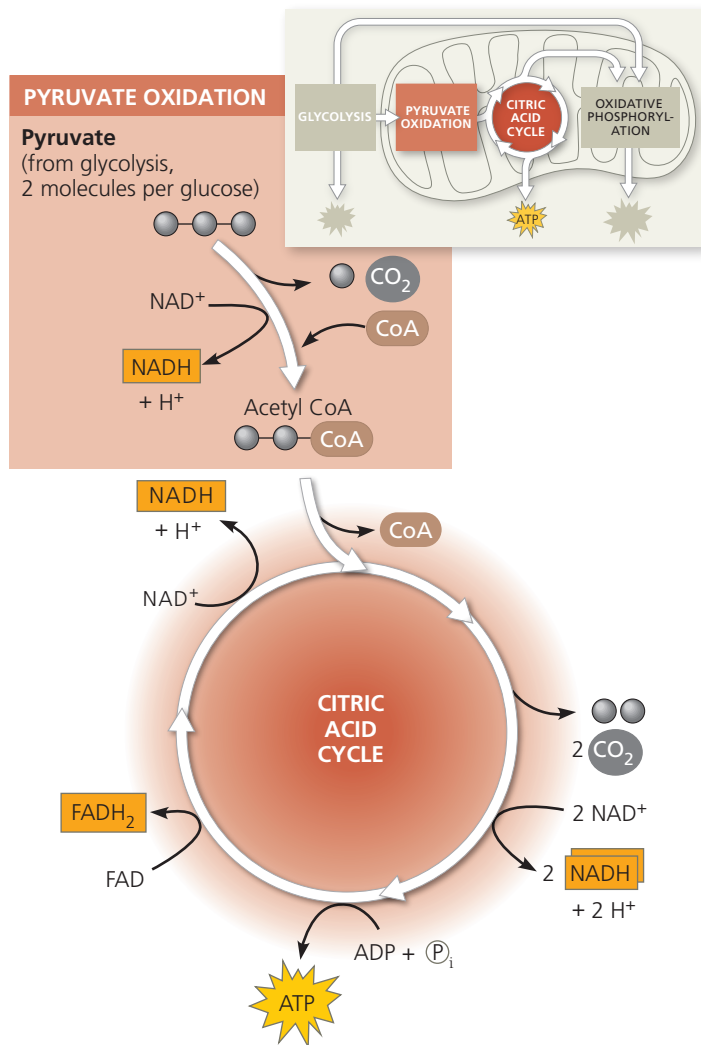
BioFlix® Animation: Glycolysis

group (—COO^-), already somewhat oxidized and thus carrying little chemical energy, is now fully oxidized and given off as a molecule of CO_2 . This is the first step in which CO_2 is released during respiration. **2** Next, the remaining two-carbon fragment is oxidized and the electrons transferred to NAD^+ , storing energy in the form of NADH . **3** Finally, coenzyme A (CoA), a sulfur-containing compound derived from a B vitamin, is attached via its sulfur atom to the two-carbon intermediate, forming acetyl CoA. Acetyl CoA has a high potential energy, which is used to transfer the acetyl group to a molecule in the citric acid cycle, a reaction that is therefore highly exergonic.

The Citric Acid Cycle

The citric acid cycle functions as a metabolic furnace that further oxidizes organic fuel derived from pyruvate. **Figure 10.11** summarizes the inputs and outputs as

✓ Figure 10.11 An overview of pyruvate oxidation and the citric acid cycle. The inputs and outputs per pyruvate molecule are shown. To calculate on a per-glucose basis, multiply by 2, because each glucose molecule is split during glycolysis into two pyruvate molecules.



Animation: The Citric Acid Cycle

pyruvate is broken down to three CO_2 molecules, including the molecule of CO_2 released during the conversion of pyruvate to acetyl CoA. The cycle generates 1 ATP per turn by substrate-level phosphorylation, but most of the chemical energy is transferred to NAD^+ and FAD during the redox reactions. The reduced coenzymes, NADH and FADH_2 , shuttle their cargo of high-energy electrons into the electron transport chain. The citric acid cycle is also called the tricarboxylic acid cycle or the Krebs cycle, the latter honoring Hans Krebs, the German-British scientist who was largely responsible for working out the pathway in the 1930s.

Now let's look at the citric acid cycle in more detail. The cycle has eight steps, each catalyzed by a specific enzyme. You can see in **Figure 10.12** that for each turn of the citric acid cycle, two carbons (red) enter in the relatively reduced form of an acetyl group (step **1**), and two different carbons (blue) leave in the completely oxidized form of CO_2 molecules (steps **3** and **4**). The acetyl group of acetyl CoA joins the cycle by combining with the compound oxaloacetate, forming citrate (step **1**). Citrate is the ionized form of citric acid, for which the cycle is named. The next seven steps decompose the citrate back to oxaloacetate. It is this regeneration of oxaloacetate that makes the process a *cycle*.

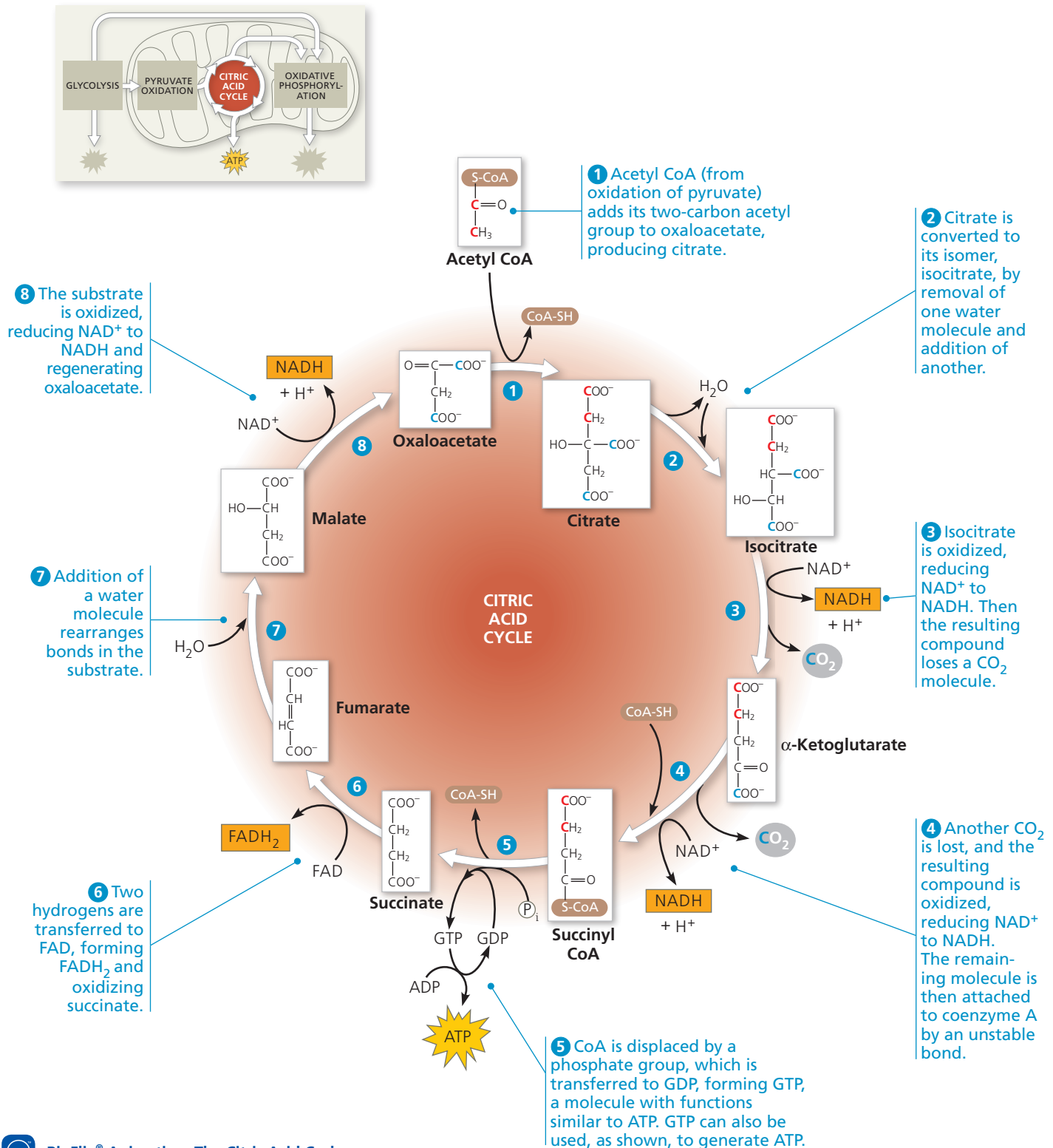
Referring to Figure 10.12, we can tally the energy-rich molecules produced by the citric acid cycle. For each acetyl group entering the cycle, 3 NAD^+ are reduced to NADH (steps **3**, **4**, and **8**). In step **6**, electrons are transferred not to NAD^+ , but to FAD , which accepts 2 electrons and 2 protons to become FADH_2 . In many animal tissue cells, the reaction in step **5** produces a guanosine triphosphate (GTP) molecule by substrate-level phosphorylation. GTP is a molecule similar to ATP in its structure and cellular function. This GTP may be used to make an ATP molecule (as shown) or directly power work in the cell. In the cells of plants, bacteria, and some animal tissues, step **5** forms an ATP molecule directly by substrate-level phosphorylation. The output from step **5** represents the only ATP generated during the citric acid cycle. Recall that each glucose gives rise to two molecules of acetyl CoA that enter the cycle. Because the numbers noted earlier are obtained from a single acetyl group entering the pathway, the total yield per glucose from the citric acid cycle turns out to be 6 NADH , 2 FADH_2 , and the equivalent of 2 ATP.

Most of the ATP produced by respiration results later, from oxidative phosphorylation, when the NADH and FADH_2 produced by the citric acid cycle and earlier steps relay the electrons extracted from food to the electron transport chain. In the process, they supply the necessary energy for the phosphorylation of ADP to ATP . We will explore this process in the next section.

▼ **Figure 10.12 A closer look at the citric acid cycle.** In the chemical structures, red type traces the fate of the two carbon atoms that enter the cycle via acetyl CoA (step 1), and blue type indicates the two carbons that exit the cycle as CO₂ in steps 3 and 4. (The red type goes only through step 5 because the succinate molecule is symmetrical; the two

ends cannot be distinguished from each other.) Notice that the carbon atoms that enter the cycle from acetyl CoA do not leave the cycle in the same turn. They remain in the cycle, occupying a different location in the molecules on their next turn, after another acetyl group is added. Therefore, the oxaloacetate regenerated at step 8 is made up of different carbon atoms

each time around. In eukaryotic cells, all the citric acid cycle enzymes are located in the mitochondrial matrix except for the enzyme that catalyzes step 6, which resides in the inner mitochondrial membrane. Carboxylic acids are represented in their ionized forms, as —COO[−], because the ionized forms prevail at the pH within the mitochondrion.



CONCEPT CHECK 10.3

1. **VISUAL SKILLS** > Name the molecules that conserve most of the energy from the redox reactions of the citric acid cycle (see Figure 10.12). How is this energy converted to a form that can be used to make ATP?
2. What processes in your cells produce the CO_2 that you exhale?
3. **VISUAL SKILLS** > The conversions shown in Figure 10.10 and step 4 of Figure 10.12 are each catalyzed by a large multienzyme complex. What similarities are there in the reactions that occur in these two cases?

For suggested answers, see Appendix A.

CONCEPT 10.4

During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis

Our main objective in this chapter is to learn how cells harvest the energy of glucose and other nutrients in food to make ATP. But the metabolic components of respiration we have examined so far, glycolysis and the citric acid cycle, produce only 4 ATP molecules per glucose molecule, all by substrate-level phosphorylation: 2 net ATP from glycolysis and 2 ATP from the citric acid cycle. At this point, molecules of NADH (and FADH_2) account for most of the energy extracted from each glucose molecule. These electron escorts link glycolysis and the citric acid cycle to the machinery of oxidative phosphorylation, which uses energy released by the electron transport chain to power ATP synthesis. In this section, you will learn first how the electron transport chain works and then how electron flow down the chain is coupled to ATP synthesis.

The Pathway of Electron Transport

The electron transport chain is a collection of molecules embedded in the inner membrane of the mitochondrion in eukaryotic cells. (In prokaryotes, these molecules reside in the plasma membrane.) The folding of the inner membrane to form cristae increases its surface area, providing space for thousands of copies of each component of the electron transport chain in a mitochondrion. The infolded membrane with its concentration of electron carrier molecules is well-suited for the series of sequential redox reactions that take place along the electron transport chain. Most components of the chain are proteins, which exist in multiprotein complexes numbered I through IV. Tightly bound to these proteins are *prosthetic groups*, nonprotein components such as cofactors and coenzymes essential for the catalytic functions of certain enzymes.

Figure 10.13 shows the sequence of electron carriers in the electron transport chain and the drop in free energy as electrons travel down the chain. During this electron transport,

Figure 10.13 Free-energy change during electron transport.

The overall energy drop (ΔG) for electrons traveling from NADH to oxygen is 53 kcal/mol, but this “fall” is broken up into a series of smaller steps by the electron transport chain. (An oxygen atom is represented here as $\frac{1}{2} \text{O}_2$ to emphasize that the electron transport chain reduces molecular oxygen, O_2 , not individual oxygen atoms.)

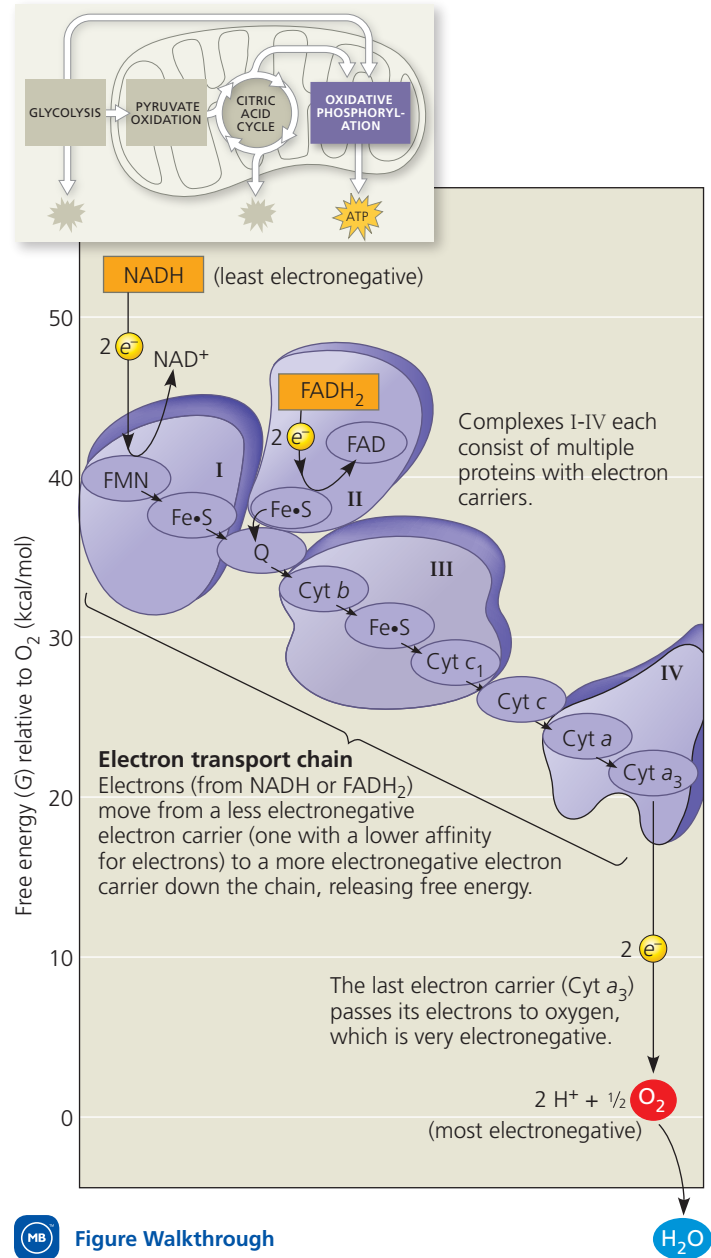


Figure Walkthrough

electron carriers alternate between reduced and oxidized states as they accept and then donate electrons. Each component of the chain becomes reduced when it accepts electrons from its “uphill” neighbor, which has a lower affinity for electrons (in other words, is less electronegative). It then returns to its oxidized form as it passes electrons to its “downhill,” more electronegative neighbor.

Now let’s take a closer look at the electrons as they drop in energy level, passing through the components of the electron transport chain in Figure 10.13. We’ll first describe the passage

of electrons through complex I in some detail as an illustration of the general principles involved in electron transport. Electrons acquired from glucose by NAD^+ during glycolysis and the citric acid cycle are transferred from NADH to the first molecule of the electron transport chain in complex I. This molecule is a flavoprotein, so named because it has a prosthetic group called flavin mononucleotide (FMN). In the next redox reaction, the flavoprotein returns to its oxidized form as it passes electrons to an iron-sulfur protein ($\text{Fe}\cdot\text{S}$ in complex I), one of a family of proteins with both iron and sulfur tightly bound. The iron-sulfur protein then passes the electrons to a compound called ubiquinone (Q in Figure 10.13). This electron carrier is a small hydrophobic molecule, the only member of the electron transport chain that is not a protein. Ubiquinone is individually mobile within the membrane rather than residing in a particular complex. (Another name for ubiquinone is coenzyme Q, or CoQ; you may have seen it sold as a nutritional supplement.)

Most of the remaining electron carriers between ubiquinone and oxygen are proteins called **cytochromes**. Their prosthetic group, called a heme group, has an iron atom that accepts and donates electrons. (The heme group in a cytochrome is similar to the heme group in hemoglobin, the protein of red blood cells, except that the iron in hemoglobin carries oxygen, not electrons.) The electron transport chain has several types of cytochromes, each named “cyt” with a letter and number to distinguish it as a different protein with a slightly different electron-carrying heme group. The last cytochrome of the chain, Cyt a_3 , passes its electrons to oxygen, which is *very* electronegative. Each oxygen atom also picks up a pair of hydrogen ions (protons) from the aqueous solution, neutralizing the -2 charge of the added electrons and forming water.

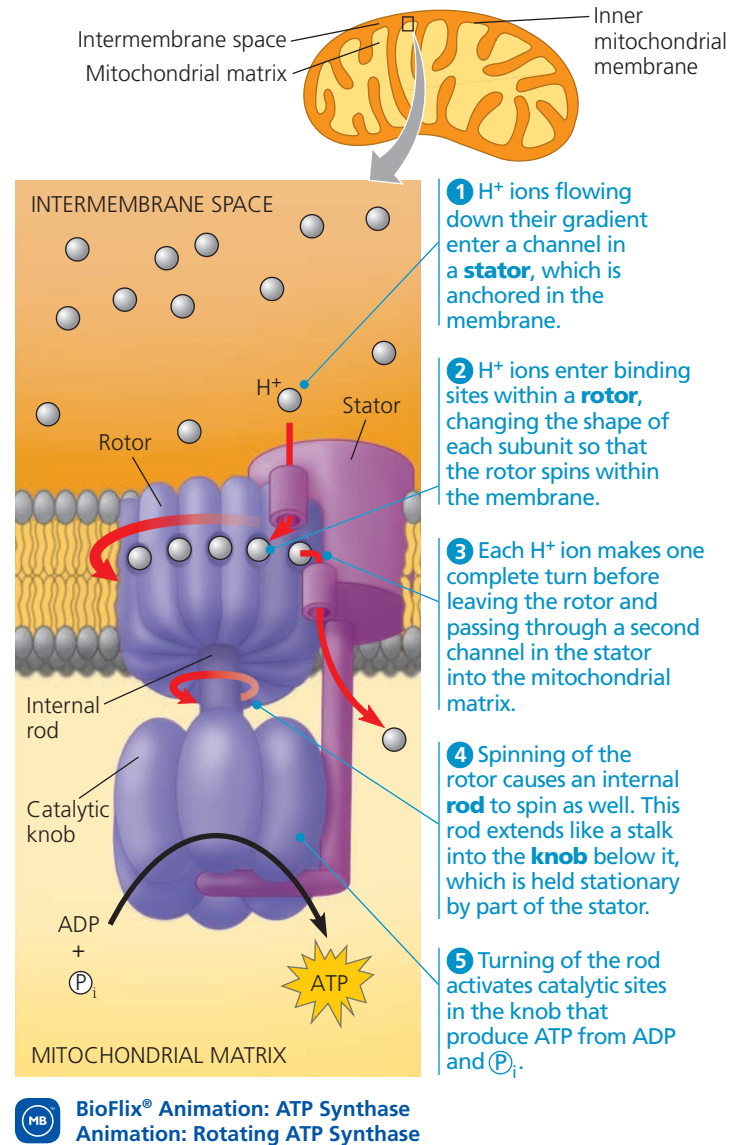
Another source of electrons for the electron transport chain is FADH_2 , the other reduced product of the citric acid cycle. Notice in Figure 10.13 that FADH_2 adds its electrons from within complex II, at a lower energy level than NADH does. Consequently, although NADH and FADH_2 each donate an equivalent number of electrons (2) for oxygen reduction, the electron transport chain provides about one-third less energy for ATP synthesis when the electron donor is FADH_2 rather than NADH . We’ll see why in the next section.

The electron transport chain makes no ATP directly. Instead, it eases the fall of electrons from food to oxygen, breaking a large free-energy drop into a series of smaller steps that release energy in manageable amounts, step by step. How does the mitochondrion (or the plasma membrane in prokaryotes) couple this electron transport and energy release to ATP synthesis? The answer is a mechanism called chemiosmosis.

Chemiosmosis: The Energy-Coupling Mechanism

Populating the inner membrane of the mitochondrion or the prokaryotic plasma membrane are many copies of a protein

▼ **Figure 10.14 ATP synthase, a molecular mill.** Multiple ATP synthases reside in eukaryotic mitochondrial and chloroplast membranes and in prokaryotic plasma membranes.



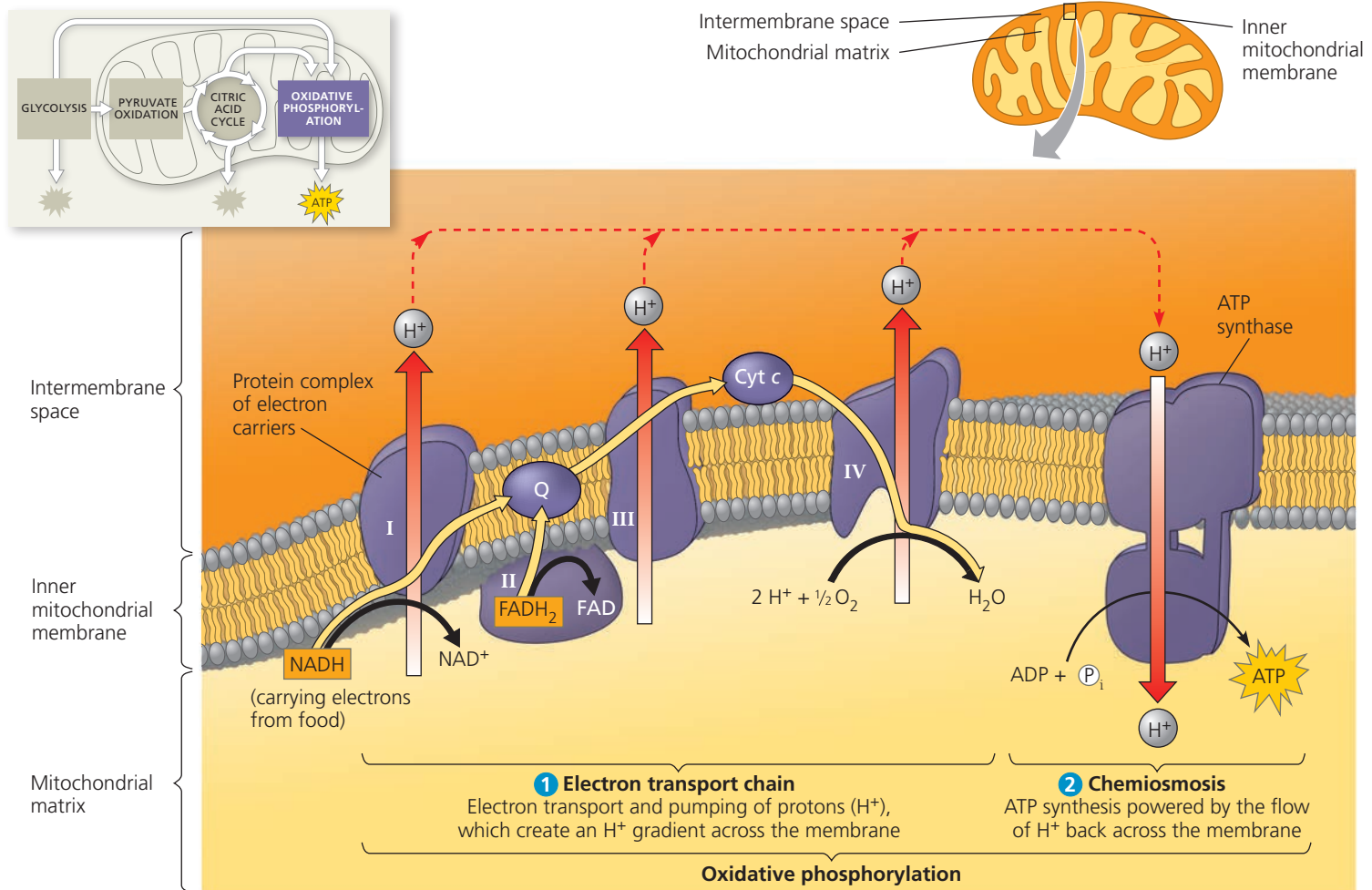
complex called **ATP synthase**, the enzyme that makes ATP from ADP and inorganic phosphate (Figure 10.14). ATP synthase works like an ion pump running in reverse. Ion pumps usually use ATP as an energy source to transport ions against their gradients. Enzymes can catalyze a reaction in either direction, depending on the ΔG for the reaction, which is affected by the local concentrations of reactants and products (see Concepts 6.2 and 6.3). Rather than hydrolyzing ATP to pump protons against their concentration gradient, under the conditions of cellular respiration ATP synthase uses the energy of an existing ion gradient to power ATP synthesis. The power source for ATP synthase is a difference in the concentration of H^+ on opposite sides of the inner mitochondrial membrane. This process, in which energy stored in the form of a hydrogen ion gradient across a membrane is used to drive cellular work such as the synthesis of ATP, is called **chemiosmosis** (from

▼ Figure 10.15 Chemiosmosis couples the electron transport chain to ATP synthesis.

1 NADH and FADH₂ shuttle high-energy electrons extracted from food during glycolysis and the citric acid cycle into an electron transport chain built into the inner mitochondrial membrane. The gold arrows trace the transport of electrons, which are finally passed to a terminal acceptor (O₂, in the case of aerobic respiration) at the “downhill” end of the chain,

forming water. Most of the electron carriers of the chain are grouped into four complexes (I–IV). Two mobile carriers, ubiquinone (Q) and cytochrome c (Cyt c), move rapidly, ferrying electrons between the large complexes. As the complexes shuttle electrons, they pump protons from the mitochondrial matrix into the intermembrane space. FADH₂ deposits its electrons via complex II and so results in fewer protons being pumped into the intermembrane

space than occurs with NADH. Chemical energy originally harvested from food is transformed into a proton-motive force, a gradient of H⁺ across the membrane. 2 During chemiosmosis, the protons flow back down their gradient via ATP synthase, which is built into the membrane nearby. The ATP synthase harnesses the proton-motive force to phosphorylate ADP, forming ATP. Together, electron transport and chemiosmosis make up oxidative phosphorylation.



WHAT IF ► If complex IV were nonfunctional, could chemiosmosis produce any ATP, and if so, how would the rate of synthesis differ?

Animation: Electron Transport
BioFlix® Animation: Electron Transport

the Greek *osmos*, push). We have previously used the word *osmosis* in discussing water transport, but here it refers to the flow of H⁺ across a membrane.

From studying the structure of ATP synthase, scientists have learned how the flow of H⁺ through this large enzyme powers ATP generation. ATP synthase is a multisubunit complex with four main parts, each made up of multiple polypeptides (see Figure 10.14). Protons move one by one into binding sites on one of the parts (the rotor), causing it to spin in a way that catalyzes ATP production from ADP and inorganic phosphate. The flow of protons thus behaves somewhat like a rushing stream that turns a waterwheel.

How does the inner mitochondrial membrane or the prokaryotic plasma membrane generate and maintain the H⁺

gradient that drives ATP synthesis by the ATP synthase protein complex? Establishing the H⁺ gradient is a major function of the electron transport chain, which is shown in its mitochondrial location in Figure 10.15. The chain is an energy converter that uses the exergonic flow of electrons from NADH and FADH₂ to pump H⁺ across the membrane, from the mitochondrial matrix into the intermembrane space. The H⁺ has a tendency to move back across the membrane, diffusing down its gradient. And the ATP synthases are the only sites that provide a route through the membrane for H⁺. As we described previously, the passage of H⁺ through ATP synthase uses the exergonic flow of H⁺ to drive the phosphorylation of ADP. Thus, the energy stored in an H⁺ gradient across a membrane couples the redox reactions of the electron transport chain to ATP synthesis.

At this point, you may be wondering how the electron transport chain pumps hydrogen ions. Researchers have found that certain members of the electron transport chain accept and release protons (H^+) along with electrons. (The aqueous solutions inside and surrounding the cell are a ready source of H^+ .) At certain steps along the chain, electron transfers cause H^+ to be taken up and released into the surrounding solution. In eukaryotic cells, the electron carriers are spatially arranged in the inner mitochondrial membrane in such a way that H^+ is accepted from the mitochondrial matrix and deposited in the intermembrane space (see Figure 10.15). The H^+ gradient that results is referred to as a **proton-motive force**, emphasizing the capacity of the gradient to perform work. The force drives H^+ back across the membrane through the H^+ channels provided by ATP synthases.

In general terms, *chemiosmosis is an energy-coupling mechanism that uses energy stored in the form of an H^+ gradient across a membrane to drive cellular work.* In mitochondria, the energy for gradient formation comes from exergonic redox reactions along the electron transport chain, and ATP synthesis is the work performed. But chemiosmosis also occurs elsewhere and in other variations. Chloroplasts use chemiosmosis to generate ATP during photosynthesis; in these organelles, light (rather than chemical energy) drives both electron flow down an electron transport chain and the resulting H^+ gradient formation. Prokaryotes, as already mentioned, generate H^+ gradients across their plasma membranes. They then tap the proton-motive force not only to make ATP inside the cell but

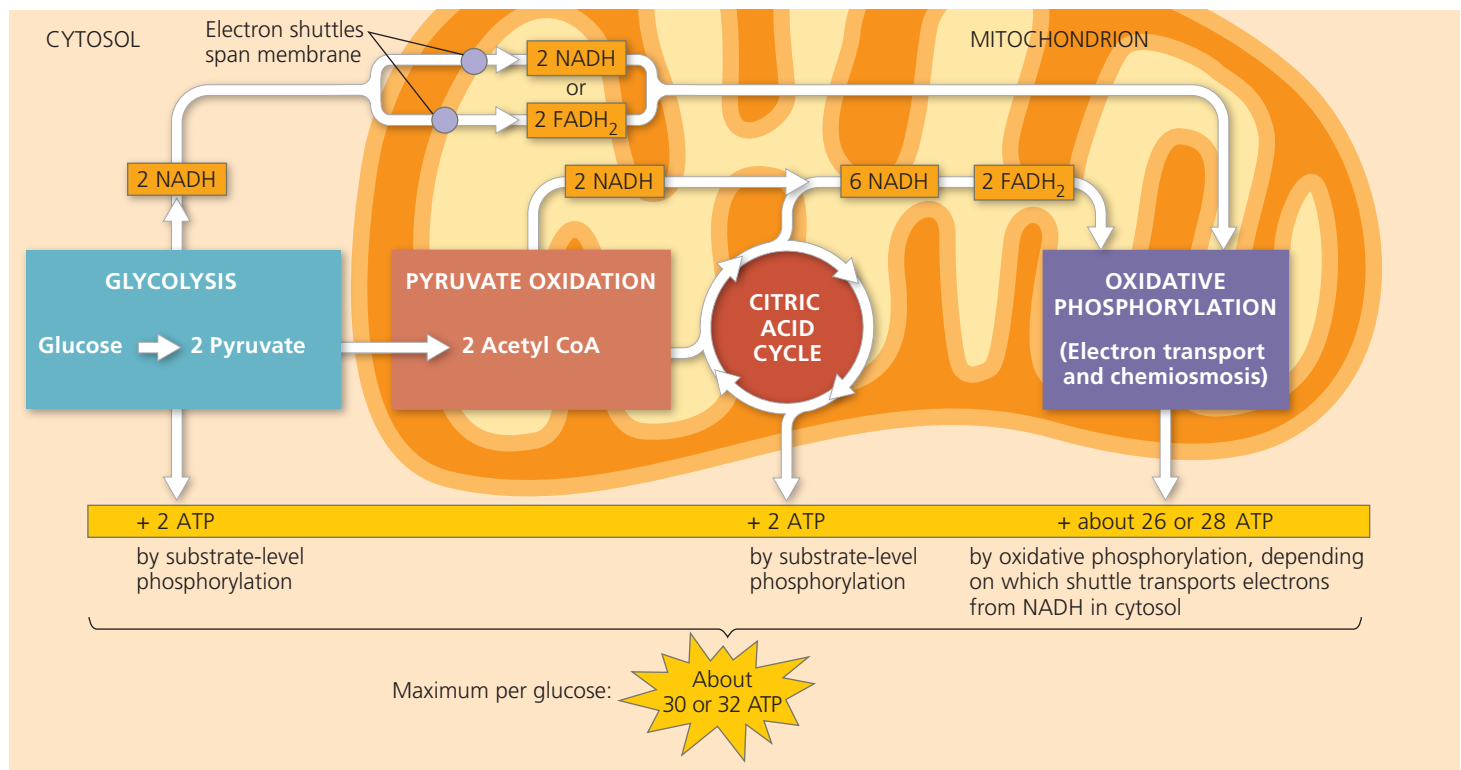
also to rotate their flagella and to pump nutrients and waste products across the membrane. Because of its central importance to energy conversions in prokaryotes and eukaryotes, chemiosmosis has helped unify the study of bioenergetics. Peter Mitchell was awarded the Nobel Prize in 1978 for originally proposing the chemiosmotic model.

An Accounting of ATP Production by Cellular Respiration

In the last few sections, we have looked rather closely at the key processes of cellular respiration. Now let's take a step back and remind ourselves of its overall function: harvesting the energy of glucose for ATP synthesis.

During respiration, most energy flows in this sequence: glucose $\rightarrow$ NADH $\rightarrow$ electron transport chain $\rightarrow$ proton-motive force $\rightarrow$ ATP. We can do some bookkeeping to calculate the ATP profit when cellular respiration oxidizes a molecule of glucose to six molecules of carbon dioxide. The three main departments of this metabolic enterprise are glycolysis, pyruvate oxidation and the citric acid cycle, and the electron transport chain, which drives oxidative phosphorylation. **Figure 10.16** gives a detailed accounting of the ATP yield for each glucose molecule that is oxidized. The tally adds the 4 ATP produced directly by substrate-level phosphorylation during glycolysis and the citric acid cycle to the many more molecules of ATP generated by oxidative

▼ **Figure 10.16** ATP yield per molecule of glucose at each stage of cellular respiration.



VISUAL SKILLS ► Explain exactly how the total of 26 or 28 ATP from oxidative phosphorylation was calculated (see the yellow bar in the figure).



Animation: ATP Yield from Cellular Respiration

phosphorylation. Each NADH that transfers a pair of electrons from glucose to the electron transport chain contributes enough to the proton-motive force to generate a maximum of about 3 ATP.

Why are the numbers in Figure 10.16 inexact? There are three reasons we cannot state an exact number of ATP molecules generated by the breakdown of one molecule of glucose. First, phosphorylation and the redox reactions are not directly coupled to each other, so the ratio of the number of NADH molecules to the number of ATP molecules is not a whole number. We know that 1 NADH results in 10 H^+ being transported out across the inner mitochondrial membrane, but the exact number of H^+ that must reenter the mitochondrial matrix via ATP synthase to generate 1 ATP has long been debated. Based on experimental data, however, most biochemists now agree that the most accurate number is 4 H^+ . Therefore, a single molecule of NADH generates enough proton-motive force for the synthesis of 2.5 ATP. The citric acid cycle also supplies electrons to the electron transport chain via $FADH_2$, but since its electrons enter later in the chain, each molecule of this electron carrier is responsible for transport of only enough H^+ for the synthesis of 1.5 ATP. These numbers also take into account the slight energetic cost of moving the ATP formed in the mitochondrion out into the cytosol, where it will be used.

Second, the ATP yield varies slightly depending on the type of shuttle used to transport electrons from the cytosol into the mitochondrion. The mitochondrial inner membrane is impermeable to NADH, so NADH in the cytosol is segregated from the machinery of oxidative phosphorylation. The 2 electrons of NADH captured in glycolysis must be conveyed into the mitochondrion by one of several electron shuttle systems. Depending on the kind of shuttle in a particular cell type, the electrons are passed either to NAD^+ or to FAD in the mitochondrial matrix (see Figures 10.15 and 10.16). If the electrons are passed to FAD, as in brain cells, only about 1.5 ATP can result from each NADH that was originally generated in the cytosol. If the electrons are passed to mitochondrial NAD^+ , as in liver cells and heart cells, the yield is about 2.5 ATP per NADH.

A third variable that reduces the yield of ATP is the use of the proton-motive force generated by the redox reactions of respiration to drive other kinds of work. For example, the proton-motive force powers the mitochondrion's uptake of pyruvate from the cytosol. However, if *all* the proton-motive force generated by the electron transport chain were used to drive ATP synthesis, one glucose molecule could generate a maximum of 28 ATP produced by oxidative phosphorylation plus 4 ATP (net) from substrate-level phosphorylation to give a total yield of about 32 ATP (or only about 30 ATP if the less efficient shuttle were functioning).

 **Animation: Electron Transport Chain: Factors Affecting ATP Yield**

We can now roughly estimate the efficiency of respiration—that is, the percentage of chemical energy in glucose that has been transferred to ATP. Recall that the complete oxidation of a mole of glucose releases 686 kcal of energy under standard conditions ($\Delta G = -686$ kcal/mol). Phosphorylation of ADP to form ATP stores at least 7.3 kcal per mole of ATP. Therefore, the efficiency of respiration is 7.3 kcal per mole of ATP times 32 moles of ATP per mole of glucose divided by 686 kcal per mole of glucose, which equals 0.34. Thus, about 34% of the potential chemical energy in glucose has been transferred to ATP; the actual percentage is bound to vary as ΔG varies under different cellular conditions. Cellular respiration is remarkably efficient in its energy conversion. By comparison, even the most efficient automobile converts only about 25% of the energy stored in gasoline to energy that moves the car.

The rest of the energy stored in glucose is lost as heat. We humans use some of this heat to maintain our relatively high body temperature (37°C), and we dissipate the rest through sweating and other cooling mechanisms.

Surprisingly, perhaps, it may be beneficial under certain conditions to reduce the efficiency of cellular respiration. A remarkable adaptation is shown by hibernating mammals, which overwinter in a state of inactivity and lowered metabolism. Although their internal body temperature is lower than normal, it still must be kept significantly higher than the external air temperature. One type of tissue, called brown fat, is made up of cells packed full of mitochondria. The inner mitochondrial membrane contains a channel protein called the uncoupling protein that allows protons to flow back down their concentration gradient without generating ATP. Activation of these proteins in hibernating mammals results in ongoing oxidation of stored fuel (fats), generating heat without any ATP production. In the absence of such an adaptation, the buildup of ATP would eventually cause cellular respiration to be shut down by regulatory mechanisms that will be discussed later. In the **Scientific Skills Exercise**, you can work with data in a related but different case where a decrease in metabolic efficiency in cells is used to generate heat.

 **BioFlix® Animation: Cellular Respiration**

CONCEPT CHECK 10.4

- WHAT IF? >** What effect would an absence of O_2 have on the process shown in Figure 10.15?
- WHAT IF? >** In the absence of O_2 , as in question 1, what do you think would happen if you decreased the pH of the intermembrane space of the mitochondrion? Explain your answer.
- MAKE CONNECTIONS >** Membranes must be fluid to function properly (as you learned in Concept 8.1). How does the operation of the electron transport chain support that assertion?

For suggested answers, see Appendix A.

SCIENTIFIC SKILLS EXERCISE

Making a Bar Graph and Evaluating a Hypothesis

Does Thyroid Hormone Level Affect Oxygen Consumption in Cells? Some animals, such as mammals and birds, maintain a relatively constant body temperature, above that of their environment, by using heat produced as a by-product of metabolism. When the core temperature of these animals drops below an internal set point, their cells are triggered to reduce the efficiency of ATP production by the electron transport chains in mitochondria. At lower efficiency, extra fuel must be consumed to produce the same number of ATPs, generating additional heat. Because this response is moderated by the endocrine system, researchers hypothesized that thyroid hormone might trigger this cellular response. In this exercise, you will use a bar graph to visualize data from an experiment that compared the metabolic rates (by measuring oxygen consumption) in mitochondria of cells from animals with different levels of thyroid hormone.

How the Experiment Was Done Liver cells were isolated from sibling rats that had low, normal, or elevated thyroid hormone levels. The oxygen consumption rate due to activity of the mitochondrial electron transport chains of each type of cell was measured under controlled conditions.

Data from the Experiment

Thyroid Hormone Level	Oxygen Consumption Rate [nmol O ₂ /(min · mg cells)]
Low	4.3
Normal	4.8
Elevated	8.7

Data from M. E. Harper and M. D. Brand, The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rats of different thyroid status, *Journal of Biological Chemistry* 268:14850–14860 (1993).

INTERPRET THE DATA

1. To visualize any differences in oxygen consumption between cell types, it will be useful to graph the data in a bar graph.



First, set up the axes. (a) What is the independent variable (intentionally varied by the researchers), which goes on the x-axis? List the categories along the x-axis; because they are discrete rather than continuous, you can list them in any order. (b) What is the dependent variable (measured by the researchers), which goes on the y-axis? (c) What units (abbreviated) should go on the y-axis? Label the y-axis, including the units specified in the data table. Determine the range of values of the data that will need to go on the y-axis. What is the largest value? Draw evenly spaced tick marks and label them, starting with 0 at the bottom.

2. Graph the data for each sample. Match each x-value with its y-value and place a mark on the graph at that coordinate, then draw a bar from the x-axis up to the correct height for each sample. Why is a bar graph more appropriate than a scatter plot or line graph? (For additional information about graphs, see the Scientific Skills Review in Appendix F.)
3. Examine your graph and look for a pattern in the data. (a) Which cell type had the highest rate of oxygen consumption, and which had the lowest? (b) Does this support the researchers' hypothesis? Explain. (c) Based on what you know about mitochondrial electron transport and heat production, predict which rats had the highest, and which had the lowest, body temperature.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

CONCEPT 10.5

Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen

Because most of the ATP generated by cellular respiration is due to the work of oxidative phosphorylation, our estimate of ATP yield from aerobic respiration depends on an adequate supply of oxygen to the cell. Without the electronegative oxygen to pull electrons down the transport chain, oxidative phosphorylation eventually ceases. However, there are two general mechanisms by which certain cells can oxidize organic fuel and generate ATP *without* the use of oxygen: anaerobic respiration and fermentation. The distinction

between these two is that an electron transport chain is used in anaerobic respiration but not in fermentation. (The electron transport chain is also called the respiratory chain because of its role in both types of cellular respiration.)

We have already mentioned anaerobic respiration, which takes place in certain prokaryotic organisms that live in environments without oxygen. These organisms have an electron transport chain but do not use oxygen as a final electron acceptor at the end of the chain. Oxygen performs this function very well because it is extremely electronegative, but other, less electronegative substances can also serve as final electron acceptors. Some “sulfate-reducing” marine bacteria, for instance, use the sulfate ion (SO₄²⁻) at the end of their respiratory chain. Operation of the chain builds up a proton-motive force used to produce ATP, but H₂S (hydrogen sulfide)

is made as a by-product rather than water. The rotten-egg odor you may have smelled while walking through a salt marsh or a mudflat signals the presence of sulfate-reducing bacteria.

Fermentation is a way of harvesting chemical energy without using either oxygen or any electron transport chain—in other words, without cellular respiration. How can food be oxidized without cellular respiration? Remember, oxidation simply refers to the loss of electrons to an electron acceptor, so it does not need to involve oxygen. Glycolysis oxidizes glucose to two molecules of pyruvate. The oxidizing agent of glycolysis is NAD^+ , and neither oxygen nor any electron transfer chain is involved. Overall, glycolysis is exergonic, and some of the energy made available is used to produce 2 ATP (net) by substrate-level phosphorylation. If oxygen is present, then additional ATP is made by oxidative phosphorylation when NADH passes electrons removed from glucose to the electron transport chain. But glycolysis generates 2 ATP whether oxygen is present or not—that is, whether conditions are aerobic or anaerobic.

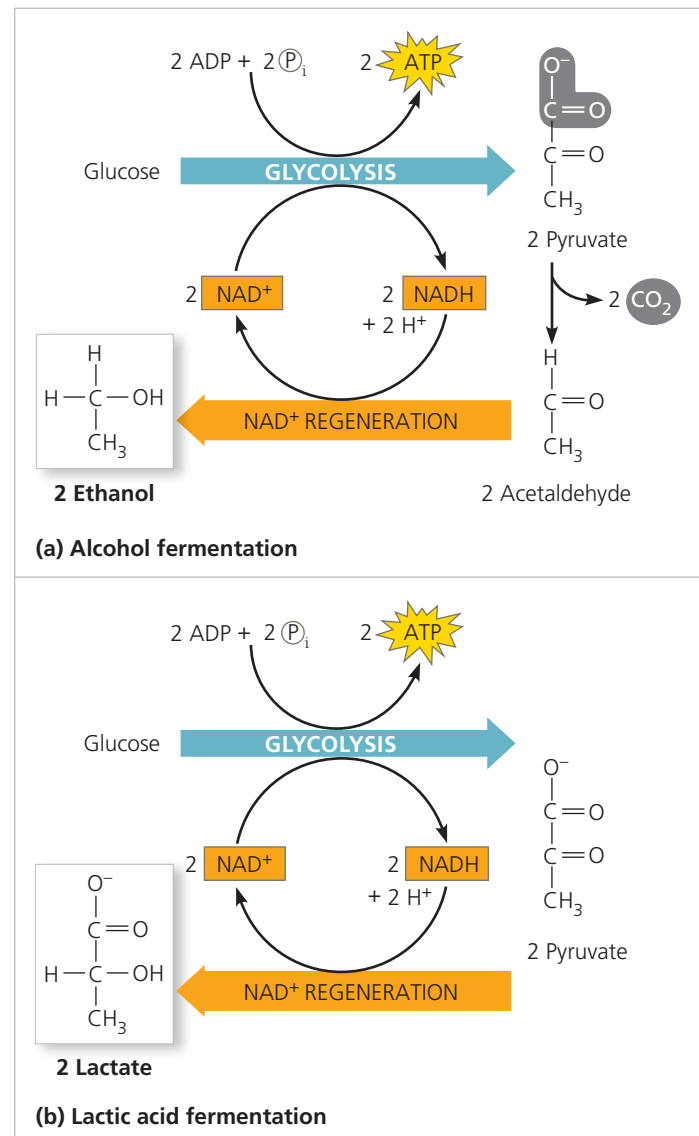
As an alternative to respiratory oxidation of organic nutrients, fermentation is an extension of glycolysis that allows continuous generation of ATP by the substrate-level phosphorylation of glycolysis. For this to occur, there must be a sufficient supply of NAD^+ to accept electrons during the oxidation step of glycolysis. Without some mechanism to recycle NAD^+ from NADH, glycolysis would soon deplete the cell's pool of NAD^+ by reducing it all to NADH and would shut itself down for lack of an oxidizing agent. Under aerobic conditions, NAD^+ is recycled from NADH by the transfer of electrons to the electron transport chain. An anaerobic alternative is to transfer electrons from NADH to pyruvate, the end product of glycolysis.

Types of Fermentation

Fermentation consists of glycolysis plus reactions that regenerate NAD^+ by transferring electrons from NADH to pyruvate or derivatives of pyruvate. The NAD^+ can then be reused to oxidize sugar by glycolysis, which nets two molecules of ATP by substrate-level phosphorylation. There are many types of fermentation, differing in the end products formed from pyruvate. Two types are alcohol fermentation and lactic acid fermentation, and both are harnessed by humans for food and industrial production.

In **alcohol fermentation** (Figure 10.17a), pyruvate is converted to ethanol (ethyl alcohol) in two steps. The first step releases carbon dioxide from the pyruvate, which is converted to the two-carbon compound acetaldehyde. In the second step, acetaldehyde is reduced by NADH to ethanol. This regenerates the supply of NAD^+ needed for the continuation of glycolysis. Many bacteria carry out alcohol fermentation under anaerobic conditions. Yeast (a fungus), in addition to aerobic respiration, also carries out alcohol fermentation. For thousands of years, humans

▼ **Figure 10.17 Fermentation.** In the absence of oxygen, many cells use fermentation to produce ATP by substrate-level phosphorylation. NAD^+ is regenerated for use in glycolysis when pyruvate, the end product of glycolysis, serves as an electron acceptor for oxidizing NADH. Two of the common end products formed from fermentation are (a) ethanol and (b) lactate, the ionized form of lactic acid.



 **Animation: Fermentation**

have used yeast in brewing, winemaking, and baking. The CO_2 bubbles generated by baker's yeast during alcohol fermentation allow bread to rise.

During **lactic acid fermentation** (Figure 10.17b), pyruvate is reduced directly by NADH to form lactate as an end product, regenerating NAD^+ with no release of CO_2 . (Lactate is the ionized form of lactic acid.) Lactic acid fermentation by certain fungi and bacteria is used in the dairy industry to make cheese and yogurt.

Human muscle cells make ATP by lactic acid fermentation when oxygen is scarce. This occurs during strenuous

exercise, when sugar catabolism for ATP production outpaces the muscle's supply of oxygen from the blood. Under these conditions, the cells switch from aerobic respiration to fermentation. The lactate that accumulates was previously thought to cause the muscle fatigue and pain that occurs a day or so after intense exercise. However, evidence shows that within an hour, blood carries the excess lactate from the muscles to the liver, where it is converted back to pyruvate by liver cells. Because oxygen is available, this pyruvate can then enter the mitochondria in liver cells and complete cellular respiration. (Next-day muscle soreness is more likely caused by trauma to small muscle fibers, which leads to inflammation and pain.)

Comparing Fermentation with Anaerobic and Aerobic Respiration

Fermentation, anaerobic respiration, and aerobic respiration are three alternative cellular pathways for producing ATP by harvesting the chemical energy of food. All three use glycolysis to oxidize glucose and other organic fuels to pyruvate, with a net production of 2 ATP by substrate-level phosphorylation. And in all three pathways, NAD^+ is the oxidizing agent that accepts electrons from food during glycolysis.

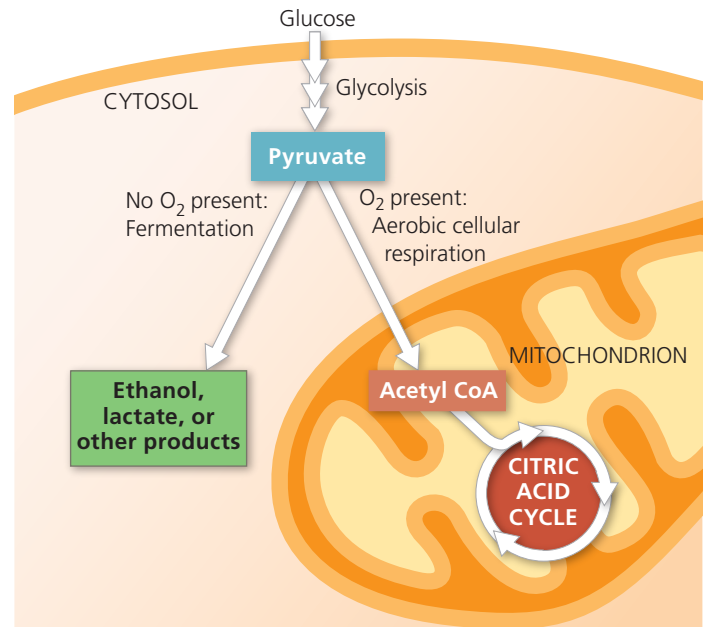
A key difference is the contrasting mechanisms for oxidizing NADH back to NAD^+ , which is required to sustain glycolysis. In fermentation, the final electron acceptor is an organic molecule such as pyruvate (lactic acid fermentation) or acetaldehyde (alcohol fermentation). In cellular respiration, by contrast, electrons carried by NADH are transferred to an electron transport chain, which regenerates the NAD^+ required for glycolysis.

Another major difference is the amount of ATP produced. Fermentation yields two molecules of ATP, produced by substrate-level phosphorylation. In the absence of an electron transport chain, the energy stored in pyruvate is unavailable. In cellular respiration, however, pyruvate is completely oxidized in the mitochondrion. Most of the chemical energy from this process is shuttled by NADH and FADH_2 in the form of electrons to the electron transport chain. There, the electrons move stepwise down a series of redox reactions to a final electron acceptor. (In aerobic respiration, the final electron acceptor is oxygen; in anaerobic respiration, the final acceptor is another molecule that is electronegative, although less so than oxygen.) Stepwise electron transport drives oxidative phosphorylation, yielding ATP. Thus, cellular respiration harvests much more energy from each sugar molecule than fermentation can. In fact, aerobic respiration yields up to 32 molecules of ATP per glucose molecule—up to 16 times as much as does fermentation.

Some organisms, called **obligate anaerobes**, carry out only fermentation or anaerobic respiration. In fact, these organisms cannot survive in the presence of oxygen, some forms of which can actually be toxic if protective systems are

▼ Figure 10.18 Pyruvate as a key juncture in catabolism.

Glycolysis is common to fermentation and cellular respiration. The end product of glycolysis, pyruvate, represents a fork in the catabolic pathways of glucose oxidation. In a facultative anaerobe or a muscle cell, which are capable of both aerobic cellular respiration and fermentation, pyruvate is committed to one of those two pathways, usually depending on whether or not oxygen is present.



not present in the cell. A few cell types, such as cells of the vertebrate brain, can carry out only aerobic oxidation of pyruvate, not fermentation. Other organisms, including yeasts and many bacteria, can make enough ATP to survive using either fermentation or respiration. Such species are called **facultative anaerobes**. On the cellular level, our muscle cells behave as facultative anaerobes. In such cells, pyruvate is a fork in the metabolic road that leads to two alternative catabolic routes (Figure 10.18). Under aerobic conditions, pyruvate can be converted to acetyl CoA, and oxidation continues in the citric acid cycle via aerobic respiration. Under anaerobic conditions, lactic acid fermentation occurs: Pyruvate is diverted from the citric acid cycle, serving instead as an electron acceptor to recycle NAD^+ . To make the same amount of ATP, a facultative anaerobe has to consume sugar at a much faster rate when fermenting than when respiring.

The Evolutionary Significance of Glycolysis

EVOLUTION The role of glycolysis in both fermentation and respiration has an evolutionary basis. Ancient prokaryotes are thought to have used glycolysis to make ATP long before oxygen was present in Earth's atmosphere. The oldest known fossils of bacteria date back 3.5 billion years, but appreciable quantities of oxygen probably did not begin to accumulate in the atmosphere until about 2.7 billion years ago. Cyanobacteria produced this O_2 as a by-product of photosynthesis. Therefore,

early prokaryotes may have generated ATP exclusively from glycolysis. The fact that glycolysis is today the most widespread metabolic pathway among Earth's organisms suggests that it evolved very early in the history of life. The cytosolic location of glycolysis also implies great antiquity; the pathway does not require any of the membrane-enclosed organelles of the eukaryotic cell, which evolved approximately 1 billion years after the first prokaryotic cell. Glycolysis is a metabolic heirloom from early cells that continues to function in fermentation and as the first stage in the breakdown of organic molecules by respiration.

CONCEPT CHECK 10.5

1. What are the end products of alcohol fermentation and lactic acid fermentation? How many molecules of ATP are generated in each of these processes?
2. **WHAT IF? >** A glucose-fed yeast cell is moved from an aerobic environment to an anaerobic one. How would its rate of glucose consumption change if ATP were to be generated at the same rate?

For suggested answers, see Appendix A.

CONCEPT 10.6

Glycolysis and the citric acid cycle connect to many other metabolic pathways

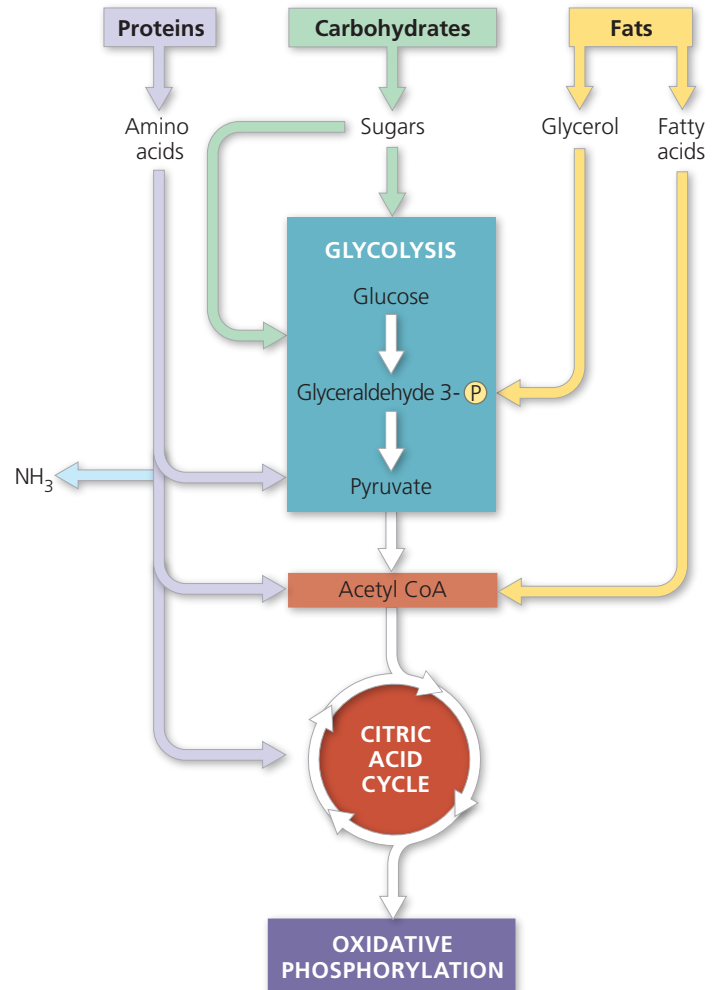
So far, we have treated the oxidative breakdown of glucose in isolation from the cell's overall metabolic economy. In this section, you will learn that glycolysis and the citric acid cycle are major intersections of the cell's catabolic (breakdown) and anabolic (biosynthetic) pathways.

The Versatility of Catabolism

Throughout this chapter, we have used glucose as an example of a fuel for cellular respiration. But free glucose molecules are not common in the diets of humans and other animals. We obtain most of our calories in the form of fats, proteins, and carbohydrates such as sucrose and other disaccharides, and starch, a polysaccharide. All these organic molecules in food can be used by cellular respiration to make ATP (**Figure 10.19**).

Glycolysis can accept a wide range of carbohydrates for catabolism. In the digestive tract, starch is hydrolyzed to glucose, which can then be broken down in the cells by glycolysis and the citric acid cycle. Similarly, glycogen, the polysaccharide that humans and many other animals store in their liver and muscle cells, can be hydrolyzed to glucose between meals as fuel for respiration. The digestion of disaccharides, including sucrose, provides glucose and other monosaccharides as fuel for respiration.

▼ **Figure 10.19 The catabolism of various molecules from food.** Carbohydrates, fats, and proteins can all be used as fuel for cellular respiration. Monomers of these molecules enter glycolysis or the citric acid cycle at various points. Glycolysis and the citric acid cycle are catabolic funnels through which electrons from all kinds of organic molecules flow on their exergonic fall to oxygen.



Proteins can also be used for fuel, but first they must be digested to their constituent amino acids. Many of the amino acids are used by the organism to build new proteins. Amino acids present in excess are converted by enzymes to intermediates of glycolysis and the citric acid cycle. Before amino acids can feed into glycolysis or the citric acid cycle, their amino groups must be removed, a process called *deamination*. The nitrogenous waste is excreted from the animal in the form of ammonia (NH_3), urea, or other waste products.

Catabolism can also harvest energy stored in fats obtained either from food or from fat cells in the body. After fats are digested to glycerol and fatty acids, the glycerol is converted to glyceraldehyde 3-phosphate, an intermediate of glycolysis. Most of the energy of a fat is stored in the fatty acids. A metabolic sequence called **beta oxidation** breaks the fatty acids down to two-carbon fragments, which enter the citric acid cycle as acetyl CoA. NADH and FADH_2 are also generated

during beta oxidation; they can enter the electron transport chain, leading to further ATP production. Fats make excellent fuels, in large part due to their chemical structure and the high energy level of their electrons (present in many C—H bonds, equally shared between carbon and hydrogen) compared to those of carbohydrates. A gram of fat oxidized by respiration produces more than twice as much ATP as a gram of carbohydrate. Unfortunately, this also means that a person trying to lose weight must work hard to use up fat stored in the body because so many calories are stockpiled in each gram of fat.

Biosynthesis (Anabolic Pathways)

Cells need substance as well as energy. Not all the organic molecules of food are destined to be oxidized as fuel to make ATP. In addition to calories, food must also provide the carbon skeletons that cells require to make their own molecules. Some organic monomers obtained from digestion can be used directly. For example, as previously mentioned, amino acids from the hydrolysis of proteins in food can be incorporated into the organism's own proteins. Often, however, the body needs specific molecules that are not present as such in food. Compounds formed as intermediates of glycolysis and the citric acid cycle can be diverted into anabolic pathways as precursors from which the cell can synthesize the molecules it requires. For example, humans can make about half of the 20 amino acids in proteins by modifying compounds siphoned away from the citric acid cycle; the rest are “essential amino acids” that must be obtained in the diet. Also, glucose can be made from pyruvate, and fatty acids can be synthesized from acetyl CoA. Of course, these anabolic, or biosynthetic, pathways do not generate ATP, but instead consume it.

In addition, glycolysis and the citric acid cycle function as metabolic interchanges that enable our cells to convert some kinds of molecules to others as we need them. For example, an intermediate compound generated during glycolysis, dihydroxyacetone phosphate (see Figure 10.9, step 5), can be converted to one of the major precursors of fats. If we eat more food than we need, we store fat even if our diet is fat-free. Metabolism is remarkably versatile and adaptable.

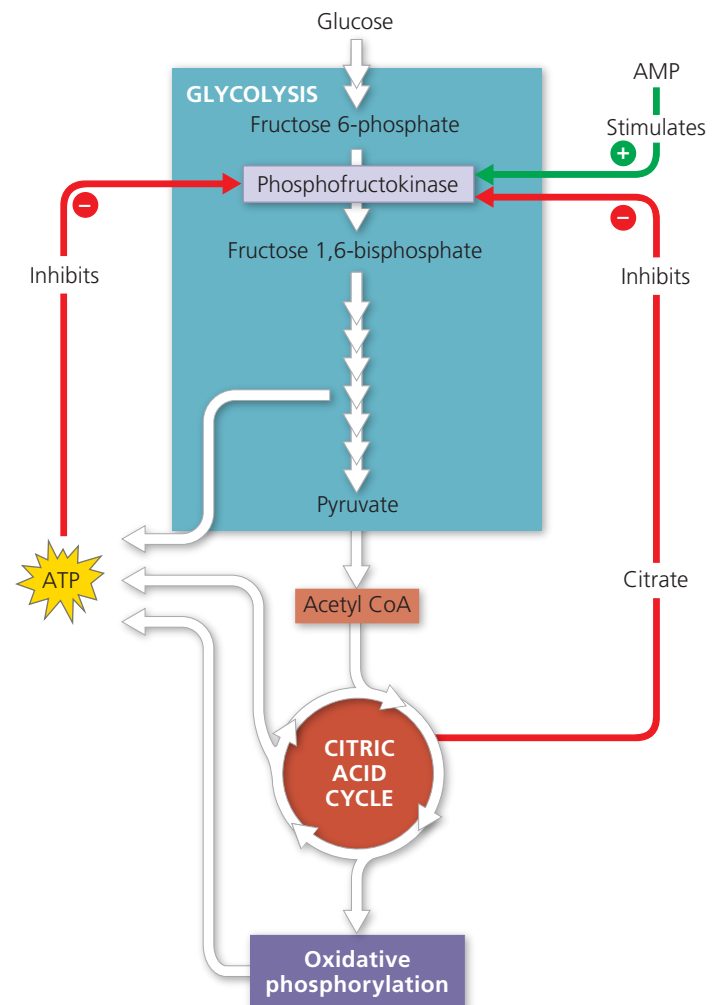
Regulation of Cellular Respiration via Feedback Mechanisms

Basic principles of supply and demand regulate the metabolic economy. The cell does not waste energy making more of a particular substance than it needs. If there is a surplus of a certain amino acid, for example, the anabolic pathway that synthesizes that amino acid from an intermediate of the citric acid cycle is switched off. The most common mechanism for this control is feedback inhibition: The end product of the anabolic pathway inhibits the enzyme that catalyzes an early step of the pathway (see Figure 6.21). This prevents the

needless diversion of key metabolic intermediates from uses that are more urgent.

The cell also controls its catabolism. If the cell is working hard and its ATP concentration begins to drop, respiration speeds up. When there is plenty of ATP to meet demand, respiration slows down, sparing valuable organic molecules for other functions. Again, control is based mainly on regulating the activity of enzymes at strategic points in the catabolic pathway. As shown in **Figure 10.20**, one important switch is phosphofructokinase, the enzyme that catalyzes step 3 of glycolysis (see Figure 10.9). That is the first step that commits the substrate irreversibly to the glycolytic pathway. By controlling the rate of this step, the cell can speed up or slow down the entire catabolic process. Phosphofructokinase can thus be considered the pacemaker of respiration.

▼ **Figure 10.20 The control of cellular respiration.** Allosteric enzymes at certain points in the respiratory pathway respond to inhibitors and activators that help set the pace of glycolysis and the citric acid cycle. Phosphofructokinase, which catalyzes an early step in glycolysis (see Figure 10.9, step 3), is one such enzyme. It is stimulated by AMP (derived from ADP) but is inhibited by ATP and by citrate. This feedback regulation adjusts the rate of respiration as the cell's catabolic and anabolic demands change.



Phosphofructokinase is an allosteric enzyme with receptor sites for specific inhibitors and activators. It is inhibited by ATP and stimulated by AMP (adenosine monophosphate), which the cell derives from ADP. As ATP accumulates, inhibition of the enzyme slows down glycolysis. The enzyme becomes active again as cellular work converts ATP to ADP (and AMP) faster than ATP is being regenerated. Phosphofructokinase is also sensitive to citrate, the first product of the citric acid cycle. If citrate accumulates in mitochondria, some of it passes into the cytosol and inhibits phosphofructokinase. This mechanism helps synchronize the rates of glycolysis and the citric acid cycle. As citrate accumulates, glycolysis slows down, and the supply of pyruvate and thus acetyl groups to the citric acid cycle decreases. If citrate consumption increases, either because of a demand for more ATP or because anabolic pathways are draining off intermediates of the citric acid cycle, glycolysis accelerates and meets the demand. Metabolic balance is augmented by the control of enzymes that catalyze other key steps of glycolysis and the citric acid cycle. Cells are thrifty, expedient, and responsive in their metabolism.

Cellular respiration and metabolic pathways play a role of central importance in organisms. Examine Figure 10.2 again to put cellular respiration into the broader context of energy flow and chemical cycling in ecosystems. The energy that keeps us alive is *released*, not *produced*, by cellular respiration. We are tapping energy that was stored in food by photosynthesis. In Chapter 11, you will learn how photosynthesis captures light and converts it to chemical energy.

CONCEPT CHECK 10.6

1. **MAKE CONNECTIONS** > Compare the structure of a fat (see Figure 5.9) with that of a carbohydrate (see Figure 5.3). What features of their structures make fat a much better fuel?
2. Under what circumstances might your body synthesize fat molecules?
3. **VISUAL SKILLS** > What will happen in a muscle cell that has used up its supply of oxygen and ATP? (Review Figures 10.18 and 10.20.)
4. **VISUAL SKILLS** > During intense exercise, can a muscle cell use fat as a concentrated source of chemical energy? Explain. (Review Figures 10.18 and 10.19.)

For suggested answers, see Appendix A.

10 Chapter Review

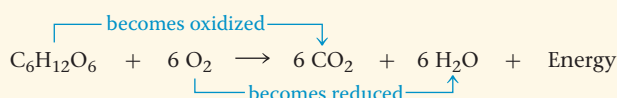
Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 10.1

Catabolic pathways yield energy by oxidizing organic fuels (pp. 237–241)

- Cells break down glucose and other organic fuels to yield chemical energy in the form of ATP. **Fermentation** is a process that results in the partial degradation of glucose without the use of oxygen. The process of **cellular respiration** is a more complete breakdown of glucose. In **aerobic respiration**, oxygen is used as a reactant; in **anaerobic respiration**, other substances are used as reactants in a similar process that harvests chemical energy without oxygen.
- The cell taps the energy stored in food molecules through **redox reactions**, in which one substance partially or totally shifts electrons to another. **Oxidation** is the total or partial loss of electrons, while **reduction** is the total or partial addition of electrons. During aerobic respiration, glucose ($C_6H_{12}O_6$) is oxidized to CO_2 , and O_2 is reduced to H_2O :



- Electrons lose potential energy during their transfer from glucose or other organic compounds to oxygen. Electrons are usually



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passed first to NAD^+ , reducing it to NADH, and are then passed from NADH to an **electron transport chain**, which conducts the electrons to O_2 in energy-releasing steps. The energy that is released is used to make ATP.

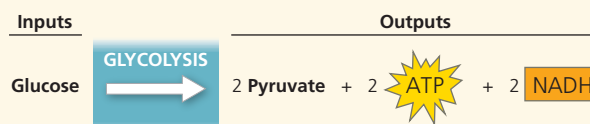
- Aerobic respiration occurs in three stages: (1) **glycolysis**, (2) pyruvate oxidation and the **citric acid cycle**, and (3) **oxidative phosphorylation** (electron transport and chemiosmosis).

? Describe the difference between the two processes in cellular respiration that produce ATP: oxidative phosphorylation and substrate-level phosphorylation.

CONCEPT 10.2

Glycolysis harvests chemical energy by oxidizing glucose to pyruvate (pp. 242–243)

- Glycolysis (“splitting of sugar”) is a series of reactions that breaks down glucose into two pyruvate molecules, which may go on to enter the citric acid cycle, and nets 2 ATP and 2 NADH per glucose molecule.

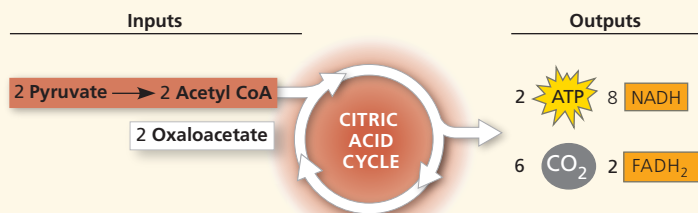


? Which reactions in glycolysis are the source of energy for the formation of ATP and NADH?

CONCEPT 10.3

After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules (pp. 243–246)

- In eukaryotic cells, pyruvate enters the mitochondrion and is oxidized to **acetyl CoA**, which is further oxidized in the citric acid cycle.

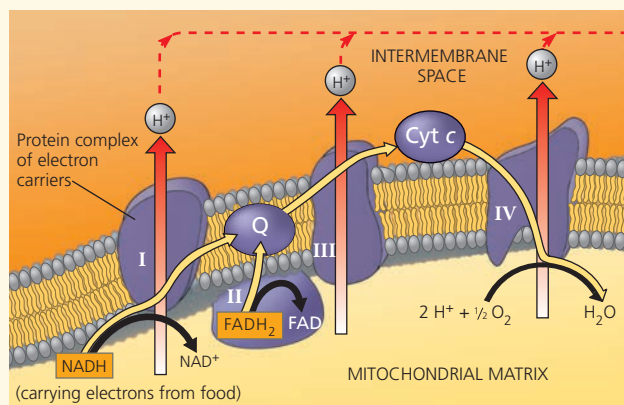


? What molecular products indicate the complete oxidation of glucose during cellular respiration?

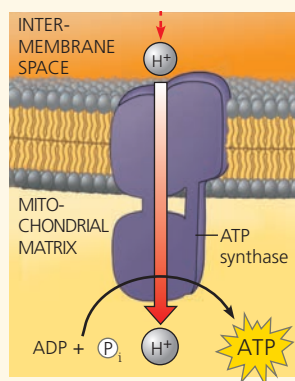
CONCEPT 10.4

During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis (pp. 246–250)

- NADH and FADH₂ transfer electrons to the electron transport chain. Electrons move down the chain, losing energy in several energy-releasing steps. Finally, electrons are passed to O₂, reducing it to H₂O.



- Along the electron transport chain, electron transfer causes protein complexes to move H⁺ from the mitochondrial matrix (in eukaryotes) to the intermembrane space, storing energy as a **proton-motive force** (H⁺ gradient). As H⁺ diffuses back into the matrix through **ATP synthase**, its passage drives the phosphorylation of ADP to form ATP, called **chemiosmosis**.
- About 34% of the energy stored in a glucose molecule is transferred to ATP during cellular respiration, producing a maximum of about 32 ATP.



? Briefly explain the mechanism by which ATP synthase produces ATP. List three locations in which ATP synthases are found.

CONCEPT 10.5

Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen (pp. 251–254)

- Glycolysis nets 2 ATP by substrate-level phosphorylation, whether oxygen is present or not. Under anaerobic conditions, anaerobic respiration or fermentation can take place. In anaerobic respiration, an electron transport chain is present with a final electron acceptor other than oxygen. In fermentation, the electrons from NADH are passed to pyruvate or a derivative of pyruvate, regenerating the NAD⁺ required to oxidize more glucose. Two common types of fermentation are **alcohol fermentation** and **lactic acid fermentation**.
- Fermentation and anaerobic or aerobic respiration all use glycolysis to oxidize glucose, but they differ in their final electron acceptor and whether an electron transport chain is used (respiration) or not (fermentation). Respiration yields more ATP; aerobic respiration, with O₂ as the final electron acceptor, yields about 16 times as much ATP as does fermentation.
- Glycolysis occurs in nearly all organisms and is thought to have evolved in ancient prokaryotes before there was O₂ in the atmosphere.

? Which process yields more ATP, fermentation or anaerobic respiration? Explain.

CONCEPT 10.6

Glycolysis and the citric acid cycle connect to many other metabolic pathways (pp. 254–256)

- Catabolic pathways funnel electrons from many kinds of organic molecules into cellular respiration. Many carbohydrates can enter glycolysis, most often after conversion to glucose. Amino acids of proteins must be deaminated before being oxidized. The fatty acids of fats undergo **beta oxidation** to two-carbon fragments and then enter the citric acid cycle as acetyl CoA. Anabolic pathways can use small molecules from food directly or build other substances using intermediates of glycolysis or the citric acid cycle.
- Cellular respiration is controlled by allosteric enzymes at key points in glycolysis and the citric acid cycle.

? Describe how the catabolic pathways of glycolysis and the citric acid cycle intersect with anabolic pathways in the metabolism of a cell.

TEST YOUR UNDERSTANDING

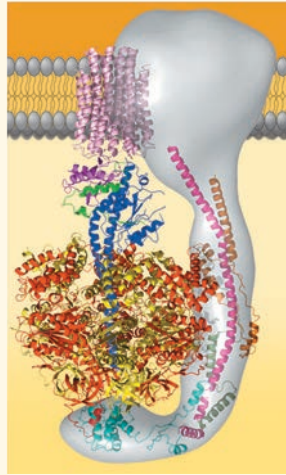
 Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. **MAKE CONNECTIONS** Step 3 in Figure 10.9 is a major point of regulation of glycolysis. The enzyme phosphofructokinase is allosterically regulated by ATP and related molecules (see Concept 6.5). Considering the overall result of glycolysis, would you expect ATP to inhibit or stimulate activity of this enzyme? Explain. (Hint: Make sure you consider the role of ATP as an allosteric regulator, not as a substrate of the enzyme.)

9. **MAKE CONNECTIONS** The proton pump shown in Figures 8.17 and 8.18 is a type of ATP synthase (see Figure 10.14). Compare the processes shown in the two figures, and say whether they are involved in active or passive transport (see Concepts 8.3 and 8.4).

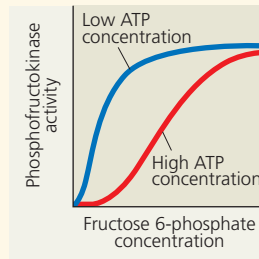


- 10. VISUAL SKILLS** This computer model shows the four parts of ATP synthase, each part consisting of a number of polypeptide subunits (the structure in gray is still an area of active research). Using Figure 10.14 as a guide, label the rotor, stator, internal rod, and catalytic knob of this molecular motor.

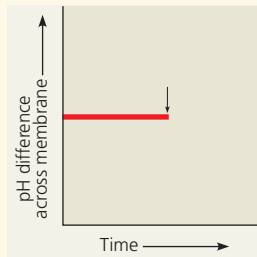


11. INTERPRET THE DATA

Phosphofructokinase is an enzyme that acts on fructose 6-phosphate at an early step in glucose breakdown. Regulation of this enzyme controls whether the sugar will continue on in the glycolytic pathway. Considering this graph, under which condition is phosphofructokinase more active? Given what you know about glycolysis and regulation of metabolism by this enzyme, explain the mechanism by which phosphofructokinase activity differs depending on ATP concentration. Explain why it makes sense that regulation of this enzyme has evolved so that it works this way.



- 12. DRAW IT** The graph here shows the pH difference across the inner mitochondrial membrane over time in an actively respiring cell. At the time indicated by the vertical arrow, a metabolic poison is added that specifically and completely inhibits all function of mitochondrial ATP synthase. Draw



what you would expect to see for the rest of the graphed line, and explain your graph.

- 13. EVOLUTION CONNECTION** ATP synthases are found in the prokaryotic plasma membrane and in mitochondria and chloroplasts. (a) Propose a hypothesis to account for an evolutionary relationship of these eukaryotic organelles and prokaryotes. (b) Explain how the amino acid sequences of the ATP synthases from the different sources could be used to support or refute your hypothesis.
- 14. SCIENTIFIC INQUIRY** In the 1930s, some physicians prescribed low doses of a compound called dinitrophenol (DNP) to help patients lose weight. This unsafe method was abandoned after some patients died. DNP uncouples the chemiosmotic machinery by making the lipid bilayer of the inner mitochondrial membrane leaky to H^+ . Explain how this could cause weight loss and death.
- 15. WRITE ABOUT A THEME: ORGANIZATION** In a short essay (100–150 words), explain how cells can harvest chemical energy with or without cellular respiration.
- 16. SYNTHESIZE YOUR KNOWLEDGE**



Coenzyme Q (CoQ) is sold as a nutritional supplement. One company uses this marketing slogan for CoQ: “Give your heart the fuel it craves most.” Considering the role of coenzyme Q, critique this claim. How do you think this product might function to benefit the heart? Is CoQ used as a “fuel” during cellular respiration?

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Photosynthetic Processes

11

▲ **Figure 11.1** How does sunlight help build the trunk, branches, and leaves of this broadleaf tree?

KEY CONCEPTS

- 11.1** Photosynthesis converts light energy to the chemical energy of food
- 11.2** The light reactions convert solar energy to the chemical energy of ATP and NADPH
- 11.3** The Calvin cycle uses the chemical energy of ATP and NADPH to reduce CO_2 to sugar
- 11.4** Alternative mechanisms of carbon fixation have evolved in hot, arid climates
- 11.5** Life depends on photosynthesis

▼ **Other organisms also benefit from photosynthesis.**



The Process That Feeds the Biosphere

Life on Earth is solar powered. Plants and other photosynthetic organisms contain cellular organelles called **chloroplasts**. Specialized molecular complexes in chloroplasts capture light energy that has traveled 150 million km from the sun and convert it to chemical energy that is stored in sugar and other organic molecules. This conversion process is called **photosynthesis**. Let's begin by placing photosynthesis in its ecological context.

Photosynthesis nourishes almost the entire living world directly or indirectly. An organism acquires the organic compounds it uses for energy and carbon skeletons by one of two major modes: autotrophic nutrition or heterotrophic nutrition. **Autotrophs** are “self-feeders” (*auto-* means “self,” and *trophos* means “feeder”); they sustain themselves without eating anything derived from other living beings. Autotrophs produce their organic molecules from CO_2 and other inorganic raw materials obtained from the environment. They are the ultimate sources of organic compounds for all nonautotrophic organisms, and for this reason, biologists refer to autotrophs as the *producers* of the biosphere.

Almost all plants are autotrophs; the only nutrients they require are water and minerals from the soil and carbon dioxide from the air. Specifically, plants are *photoautotrophs*, organisms that use light as a source of energy to synthesize organic substances (**Figure 11.1**). Photosynthesis also occurs in algae, certain

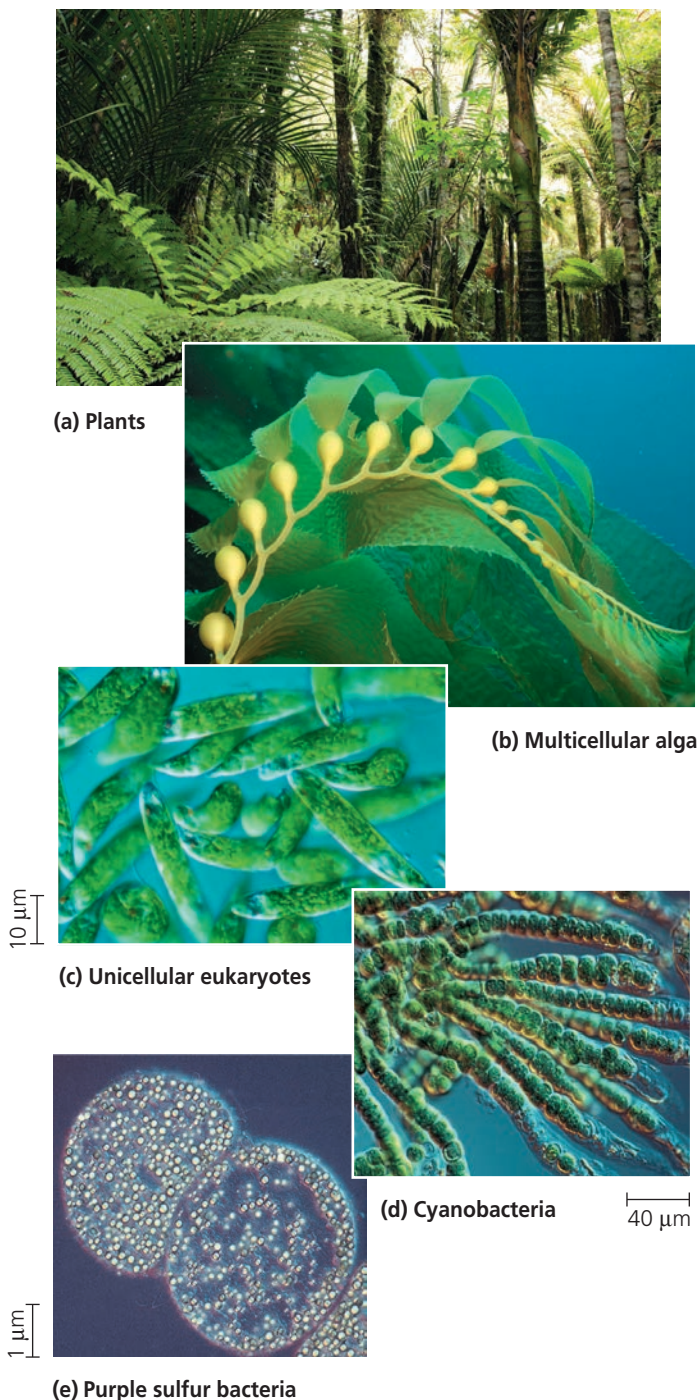
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Get Ready for This Chapter

unicellular eukaryotes, and some prokaryotes (**Figure 11.2**). In this chapter, we will touch on these other groups in passing, but our emphasis will be on plants. Variations in autotrophic nutrition that occur in prokaryotes and algae will be described in Concept 27.3.

▼ **Figure 11.2 Photoautotrophs.** These organisms use light energy to drive the synthesis of organic molecules from carbon dioxide and (in most cases) water. They feed themselves and the entire living world. On land, **(a)** plants are the predominant producers of food. In aquatic environments, photoautotrophs include unicellular and **(b)** multicellular algae, such as this kelp; **(c)** some non-algal unicellular eukaryotes, such as *Euglena*; **(d)** the prokaryotes called cyanobacteria; and **(e)** other photosynthetic prokaryotes, such as these purple sulfur bacteria, which produce sulfur (the yellow globules within the cells) (c–e, LMs).



Heterotrophs obtain organic material by the second major mode of nutrition. Unable to make their own food, they live on compounds produced by other organisms (*hetero*-means “other”). Heterotrophs are the biosphere’s *consumers*. The most obvious “other-feeding” occurs when an animal eats plants or other organisms. But heterotrophic nutrition may be more subtle. Some heterotrophs consume the remains of other organisms by decomposing and feeding on organic litter such as dead organisms, feces, and fallen leaves; these types of heterotrophs are known as *decomposers*. Most fungi and many types of prokaryotes get their nourishment this way. Almost all heterotrophs, including humans, are completely dependent, either directly or indirectly, on photoautotrophs for food—and also for oxygen, a by-product of photosynthesis.

 **BioFlix® Animation: The Flow of Carbon Atoms in Producers, Consumers, and Decomposers**

The Earth’s supply of fossil fuels was formed from remains of organisms that died hundreds of millions of years ago. In a sense, then, fossil fuels represent stores of the sun’s energy from the distant past. Because these resources are being used at a much higher rate than they are replenished, researchers are exploring methods of capitalizing on the photosynthetic process to provide alternative fuels (**Figure 11.3**).

In this chapter, you’ll learn how photosynthesis works. After discussing general principles of photosynthesis, we’ll consider the two stages of photosynthesis: the light reactions, which capture solar energy and transform it into chemical energy; and the Calvin cycle, which uses that chemical

▼ **Figure 11.3 Alternative fuels from algae.** The power of sunlight can be tapped to generate a sustainable alternative to fossil fuels. Species of unicellular algae that are prolific producers of plant oils can be cultured in long, transparent tanks called photobioreactors, such as the one shown here at Arizona State University. A simple chemical process can yield “biodiesel,” which can be mixed with gasoline or used alone to power vehicles.



WHAT IF? ► The main product of fossil fuel combustion is CO_2 , and this is the source of the increase in atmospheric CO_2 concentration. Scientists have proposed strategically situating containers of these algae near industrial plants or near highly congested city streets. Considering the process of photosynthesis, how does this arrangement make sense?

 **ABC News Video: Turning Algae into Biofuel**

energy to make the organic molecules of food. Finally, we will consider some aspects of photosynthesis from an evolutionary perspective.

CONCEPT 11.1

Photosynthesis converts light energy to the chemical energy of food

The remarkable ability of an organism to harness light energy and use it to drive the synthesis of organic compounds emerges from structural organization in the cell: Photosynthetic enzymes and other molecules are grouped together in a biological membrane, enabling the necessary series of chemical reactions to be carried out efficiently. The process of photosynthesis most likely originated in a group of bacteria that had infolded regions of the plasma membrane containing clusters of such molecules. In existing photosynthetic bacteria, infolded photosynthetic membranes function similarly to the internal membranes of the chloroplast, a eukaryotic organelle. According to what has come to be known as the endosymbiont theory, the original chloroplast was a photosynthetic prokaryote that lived inside an ancestor of eukaryotic cells. (You learned about this theory in Concept 7.5, and it will be described more fully in Concept 25.3.) Chloroplasts are present in a variety of photosynthesizing organisms (see some examples in Figure 11.2), but here we focus on chloroplasts in plants.

Chloroplasts: The Sites of Photosynthesis in Plants

All green parts of a plant, including green stems and unripened fruit, have chloroplasts, but the leaves are the major sites of photosynthesis in most plants (Figure 11.4). There are about half a million chloroplasts in a chunk of leaf with a top surface area of 1 mm². Chloroplasts are found mainly in the cells of the **mesophyll**, the tissue in the interior of the leaf. Carbon dioxide enters the leaf, and oxygen exits, by way of microscopic pores called **stomata** (singular, *stoma*; from the Greek, meaning “mouth”). Water absorbed by the roots is delivered to the leaves in veins. Leaves also use veins to export sugar to roots and other nonphotosynthetic parts of the plant.

A typical mesophyll cell has about 30–40 chloroplasts, each measuring about 2–4 μm by 4–7 μm. A chloroplast has two membranes surrounding a dense fluid called the **stroma**. Suspended within the stroma is a third membrane system, made up of sacs called **thylakoids**, which segregates the stroma from the *thylakoid space* inside these sacs. In some places, thylakoid sacs are stacked in columns called **grana** (singular, *granum*). **Chlorophyll**, the green pigment that gives leaves their color, resides in the thylakoid membranes of the chloroplast. (The internal photosynthetic membranes

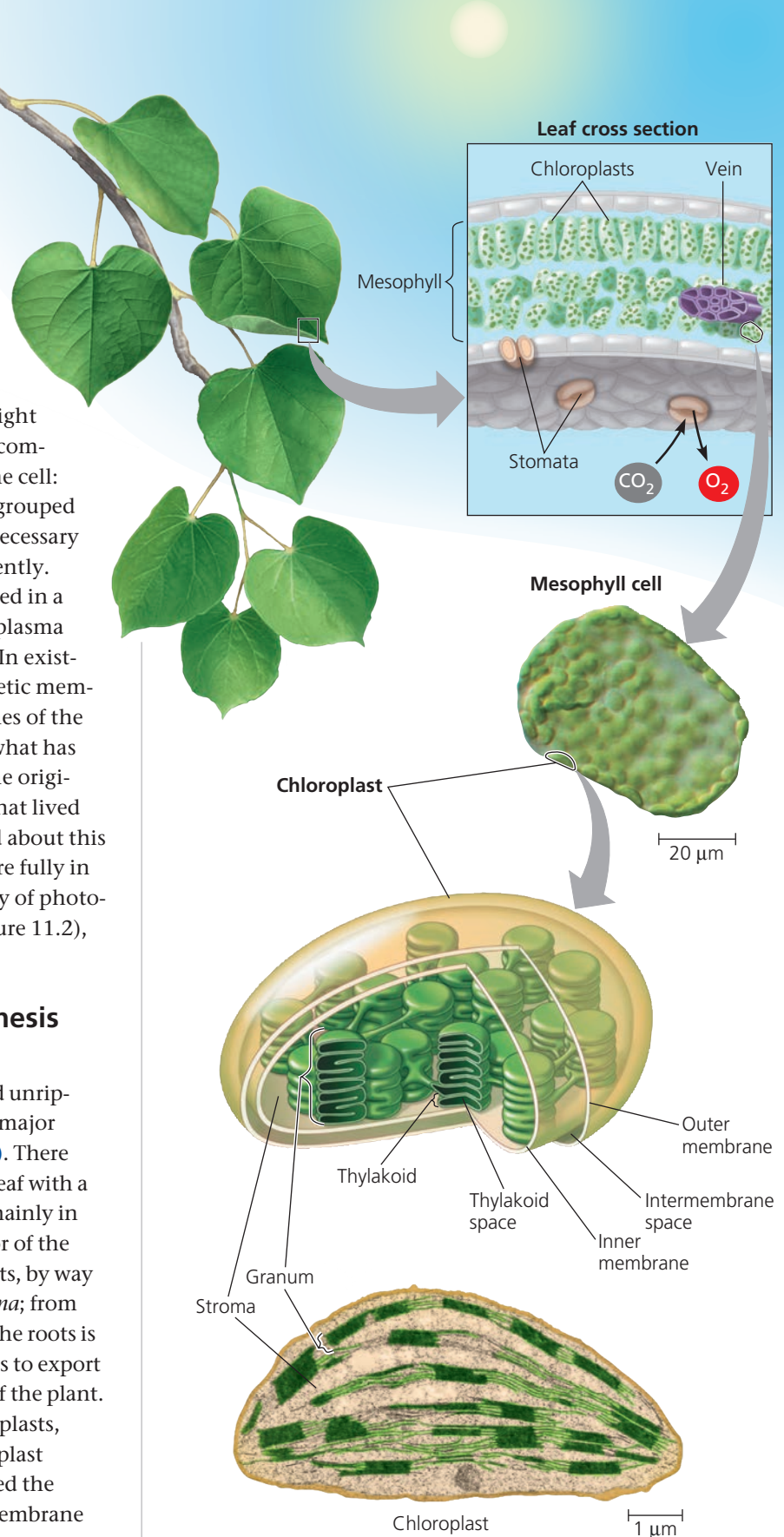


Figure 11.4 Zooming in on the location of photosynthesis in a plant. Leaves are the major organs of photosynthesis in plants. These images take you into a leaf, then into a cell, and finally into a chloroplast, the organelle where photosynthesis occurs (middle, LM; bottom, TEM).



Video: Chloroplasts in Motion

of some prokaryotes are also called thylakoid membranes; see Figure 27.8b.) It is the light energy absorbed by chlorophyll that drives the synthesis of organic molecules in the chloroplast. Now that we have looked at the sites of photosynthesis in plants, we are ready to look more closely at the process of photosynthesis.

Tracking Atoms Through Photosynthesis: Scientific Inquiry

Scientists have tried for centuries to piece together the process by which plants make food. Although some of the steps are still not completely understood, the overall photosynthetic equation has been known since the 1800s: In the presence of light, the green parts of plants produce organic compounds and oxygen from carbon dioxide and water. Using molecular formulas, we can summarize the complex series of chemical reactions in photosynthesis with this chemical equation:

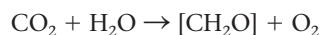


We use glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) here to simplify the relationship between photosynthesis and respiration, but the direct product of photosynthesis is actually a three-carbon sugar that can be used to make glucose. Water appears on both sides of the equation because 12 molecules are consumed and 6 molecules are newly formed during photosynthesis. We can simplify the equation by indicating only the net consumption of water:



Writing the equation in this form, we can see that the overall chemical change during photosynthesis is the reverse of the one that occurs during cellular respiration (see Concept 10.1). Both of these metabolic processes occur in plant cells. However, as you will soon learn, chloroplasts do not synthesize sugars by simply reversing the steps of respiration.

Now let's divide the photosynthetic equation by 6 to put it in its simplest possible form:



Here, the brackets indicate that CH_2O is not an actual sugar but represents the general formula for a carbohydrate (see Concept 5.2). In other words, we are imagining the synthesis of a sugar molecule one carbon at a time (with six repetitions theoretically adding up to a glucose molecule— $\text{C}_6\text{H}_{12}\text{O}_6$). Let's now see how researchers tracked the elements C, H, and O from the reactants of photosynthesis to the products.

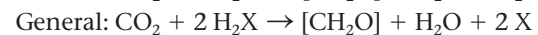
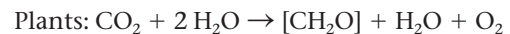
The Splitting of Water

One of the first clues to the mechanism of photosynthesis came from the discovery that the O_2 given off by plants is derived from H_2O and not from CO_2 . The chloroplast splits water into hydrogen and oxygen. Before this discovery,

the prevailing hypothesis was that photosynthesis split carbon dioxide ($\text{CO}_2 \rightarrow \text{C} + \text{O}_2$) and then added water to the carbon ($\text{C} + \text{H}_2\text{O} \rightarrow [\text{CH}_2\text{O}]$). This hypothesis predicted that the O_2 released during photosynthesis came from CO_2 . This idea was challenged in the 1930s by C. B. van Niel, of Stanford University. Van Niel was investigating photosynthesis in bacteria that make their carbohydrate from CO_2 but do not release O_2 . He concluded that, at least in these bacteria, CO_2 is not split into carbon and oxygen. One group of bacteria used hydrogen sulfide (H_2S) rather than water for photosynthesis, forming yellow globules of sulfur as a waste product (these globules are visible in Figure 11.2e). Here is the chemical equation for photosynthesis in these sulfur bacteria:

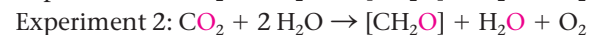


Van Niel reasoned that the bacteria split H_2S and used the hydrogen atoms to make sugar. He then generalized that idea, proposing that all photosynthetic organisms require a hydrogen source but that the source varies:



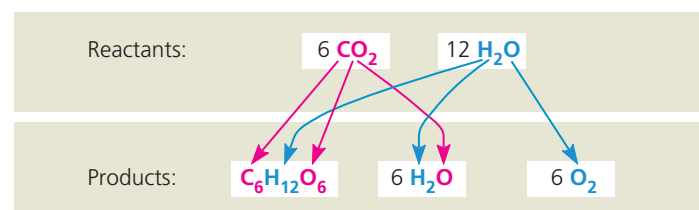
Thus, van Niel hypothesized that plants split H_2O as a source of electrons from hydrogen atoms, releasing O_2 as a by-product.

Nearly 20 years later, scientists confirmed van Niel's hypothesis by using oxygen-18 (^{18}O), a heavy isotope, as a tracer to follow the path of oxygen atoms during photosynthesis. The experiments showed that the O_2 from plants was labeled with ^{18}O only if water was the source of the tracer (experiment 1). If the ^{18}O was introduced to the plant in the form of CO_2 , the label did not turn up in the released O_2 (experiment 2). In the following summary, magenta denotes labeled atoms of oxygen (^{18}O):



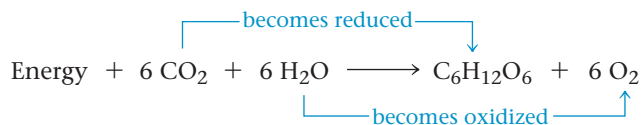
A significant result of the shuffling of atoms during photosynthesis is the extraction of hydrogen from water and its incorporation into sugar. The waste product of photosynthesis, O_2 , is released to the atmosphere. **Figure 11.5** shows the fates of all atoms in photosynthesis.

Figure 11.5 Tracking atoms through photosynthesis. The atoms from CO_2 are shown in magenta, and the atoms from H_2O are shown in blue.



Photosynthesis as a Redox Process

Let's briefly compare photosynthesis with cellular respiration. Both processes involve redox reactions. During cellular respiration, energy is released from sugar when electrons associated with hydrogen are transported by carriers to oxygen, forming water as a by-product (see Concept 10.1). The electrons lose potential energy as they “fall” down the electron transport chain toward electronegative oxygen, and the mitochondrion harnesses that energy to synthesize ATP (see Figure 10.15). Photosynthesis reverses the direction of electron flow. Water is split, and its electrons are transferred along with hydrogen ions (H^+) from the water to carbon dioxide, reducing it to sugar.



Because the electrons increase in potential energy as they move from water to sugar, this process requires energy—in other words, it is endergonic. This energy boost that occurs during photosynthesis is provided by light.

The Two Stages of Photosynthesis: A Preview

The equation for photosynthesis is a deceptively simple summary of a very complex process. Actually, photosynthesis is not a single process, but two processes, each with multiple steps. These two stages of photosynthesis are known as the **light reactions** (the *photo* part of photosynthesis) and the **Calvin cycle** (the *synthesis* part) (Figure 11.6).

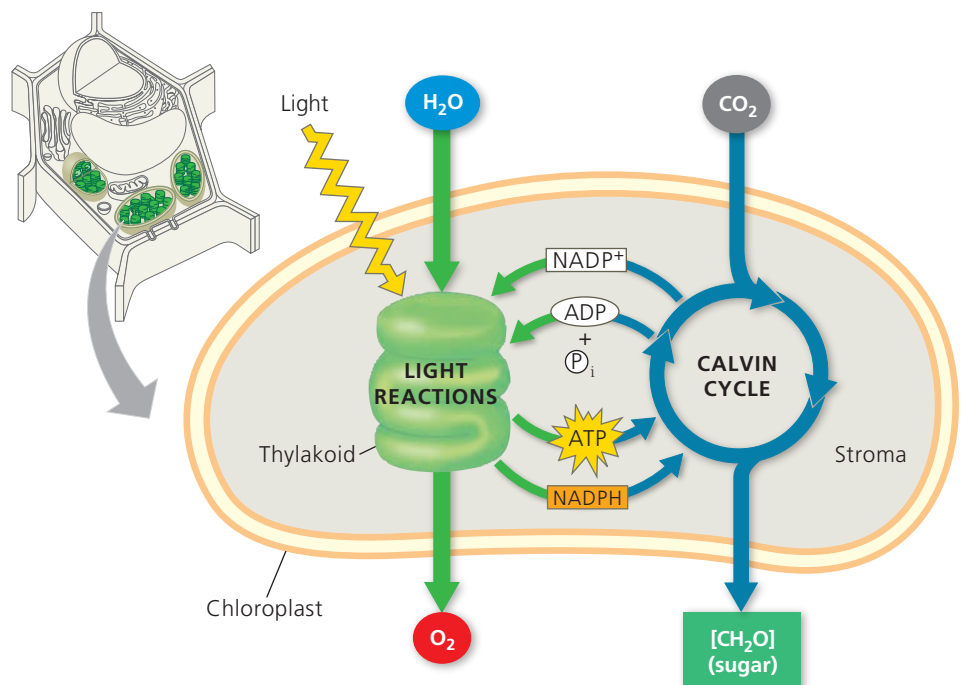
The light reactions are the steps of photosynthesis that convert solar energy to chemical energy. Water is split, providing

a source of electrons and protons (hydrogen ions, H^+) and giving off O_2 as a by-product. Light absorbed by chlorophyll drives a transfer of the electrons and hydrogen ions from water to an acceptor called **NADP⁺** (nicotinamide adenine dinucleotide phosphate), where they are temporarily stored. (The electron acceptor NADP⁺ is first cousin to NAD⁺, which functions as an electron carrier in cellular respiration; the two molecules differ only by the presence of an extra phosphate group in the NADP⁺ molecule.) The light reactions use solar energy to reduce NADP⁺ to **NADPH** by adding a pair of electrons along with an H^+ . The light reactions also generate ATP, using chemiosmosis to power the addition of a phosphate group to ADP, a process called **photophosphorylation**. Thus, light energy is initially converted to chemical energy in the form of two compounds: NADPH and ATP. NADPH, a source of electrons, acts as “reducing power” that can be passed along to an electron acceptor, reducing it, while ATP is the versatile energy currency of cells. Notice that the light reactions produce no sugar; that happens in the second stage of photosynthesis, the Calvin cycle.

The Calvin cycle is named for Melvin Calvin, who, along with his colleagues James Bassham and Andrew Benson, began to elucidate its steps in the late 1940s. The cycle begins by incorporating CO_2 from the air into organic molecules already present in the chloroplast. This initial incorporation of carbon into organic compounds is known as **carbon fixation**. The Calvin cycle then reduces the fixed carbon to carbohydrate by the addition of electrons. The reducing power is provided by NADPH, which acquired its cargo of electrons in the light reactions. To convert CO_2 to carbohydrate, the Calvin cycle also requires chemical energy

► **Figure 11.6 An overview of photosynthesis: cooperation of the light reactions and the Calvin cycle.** In the chloroplast, the thylakoid membranes (green) are the sites of the light reactions, whereas the Calvin cycle occurs in the stroma (gray). The light reactions use solar energy to make ATP and NADPH, which supply chemical energy and reducing power, respectively, to the Calvin cycle. The Calvin cycle incorporates CO_2 into organic molecules, which are converted to sugar. (Recall that most simple sugars have formulas that are some multiple of CH_2O .) To visualize these processes in their cellular context, see Figure 7.32.

 **BioFlix® Animation: Photosynthesis**



in the form of ATP, which is also generated by the light reactions. Thus, it is the Calvin cycle that makes sugar, but it can do so only with the help of the NADPH and ATP produced by the light reactions. The metabolic steps of the Calvin cycle are sometimes referred to as the dark reactions, or light-independent reactions, because none of the steps requires light *directly*. Nevertheless, the Calvin cycle in most plants occurs during daylight, for only then can the light reactions provide the NADPH and ATP that the Calvin cycle requires. In essence, the chloroplast uses light energy to make sugar by coordinating the two stages of photosynthesis.

As Figure 11.6 indicates, the thylakoids of the chloroplast are the sites of the light reactions, while the Calvin cycle occurs in the stroma. On the outside of the thylakoids, molecules of NADP⁺ and ADP pick up electrons and phosphate, respectively, and NADPH and ATP are then released to the stroma, where they play crucial roles in the Calvin cycle. The two stages of photosynthesis are treated in this figure as metabolic modules that take in ingredients and crank out products. In the next two sections, we'll look more closely at how the two stages work, beginning with the light reactions.

CONCEPT CHECK 11.1

1. **MAKE CONNECTIONS** ➤ How do the CO₂ molecules used in photosynthesis reach and enter the chloroplasts inside leaf cells? (See Concept 8.2.)
2. Explain how the use of an oxygen isotope helped elucidate the chemistry of photosynthesis.
3. **WHAT IF?** ➤ The Calvin cycle requires ATP and NADPH, products of the light reactions. If a classmate asserted that the light reactions don't depend on the Calvin cycle and, with continual light, could just keep on producing ATP and NADPH, how would you respond?

For suggested answers, see Appendix A.

CONCEPT 11.2

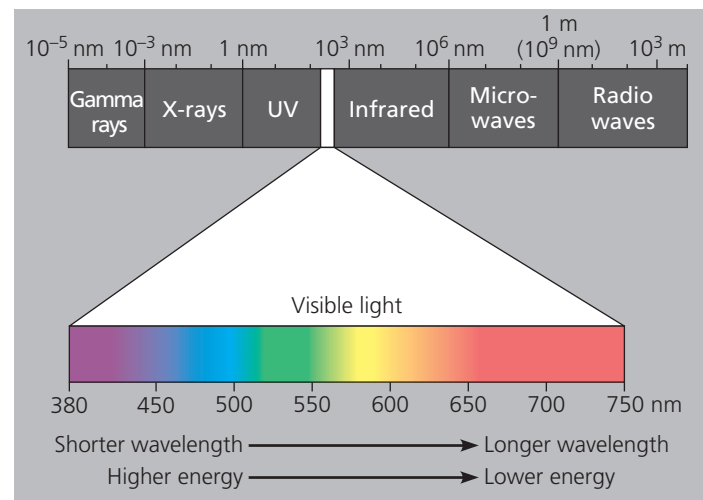
The light reactions convert solar energy to the chemical energy of ATP and NADPH

Chloroplasts are chemical factories powered by the sun. Their thylakoids transform light energy into the chemical energy of ATP and NADPH, which will be used to synthesize glucose and other molecules that can be used as energy sources. To better understand the conversion of light to chemical energy, we need to know about some important properties of light.

The Nature of Sunlight

Light is a form of energy known as electromagnetic energy, also called electromagnetic radiation. Electromagnetic energy travels in rhythmic waves analogous to those created by

▼ **Figure 11.7 The electromagnetic spectrum.** White light is a mixture of all wavelengths of visible light. A prism can sort white light into its component colors by bending light of different wavelengths at different angles. (Droplets of water in the atmosphere can act as prisms, causing a rainbow to form.) Visible light drives photosynthesis.



dropping a pebble into a pond. Electromagnetic waves, however, are disturbances of electric and magnetic fields rather than disturbances of a material medium such as water.

The distance between the crests of electromagnetic waves is called the **wavelength**. Wavelengths range from less than a nanometer (for gamma rays) to more than a kilometer (for radio waves). This entire range of radiation is known as the **electromagnetic spectrum** (Figure 11.7). The segment most important to life is the narrow band from about 380 nm to 750 nm in wavelength. This radiation is known as **visible light** because it can be detected as various colors by the human eye.

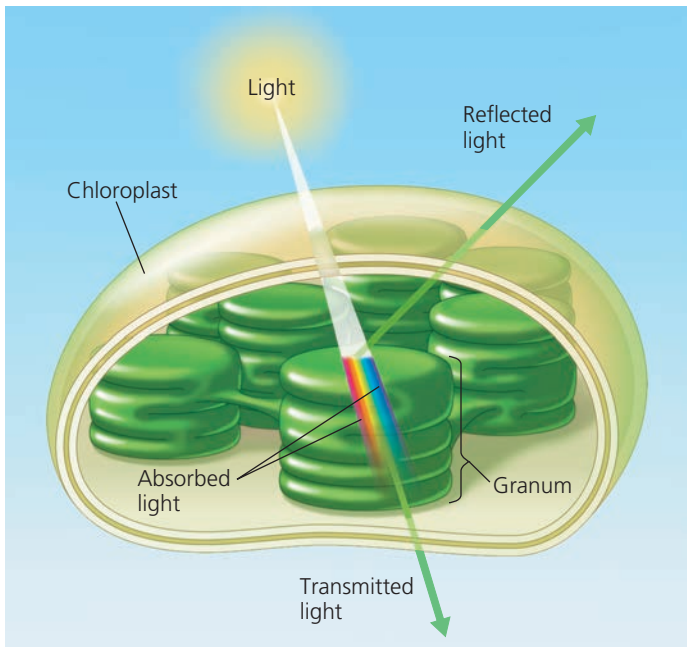
The model of light as waves explains many of light's properties, but in certain respects light behaves as though it consists of discrete particles, called **photons**. Photons are not tangible objects, but they act like objects in that each of them has a fixed quantity of energy. The amount of energy is inversely related to the wavelength of the light: The shorter the wavelength, the greater the energy of each photon of that light. Thus, a photon of violet light packs nearly twice as much energy as a photon of red light (see Figure 11.7).

Although the sun radiates the full spectrum of electromagnetic energy, the atmosphere acts like a selective window, allowing visible light to pass through while screening out a substantial fraction of other radiation. The part of the spectrum we can see—visible light—is also the radiation that drives photosynthesis.

Photosynthetic Pigments: The Light Receptors

When light meets matter, it may be reflected, transmitted, or absorbed. Substances that absorb visible light are known as

▼ **Figure 11.8 Why leaves are green: interaction of light with chloroplasts.** The chlorophyll molecules of chloroplasts absorb violet-blue and red light (the colors most effective in driving photosynthesis) and reflect or transmit green light. This is why leaves appear green.



Animation: Light Energy and Pigments

pigments. Different pigments absorb light of different wavelengths, and the wavelengths that are absorbed disappear. If a pigment is illuminated with white light, the color we see is the color most reflected or transmitted by the pigment. (If a pigment absorbs all wavelengths, it appears black.) We see green when we look at a leaf because chlorophyll absorbs violet-blue and red light while transmitting and reflecting green light (**Figure 11.8**). The ability of a pigment to absorb various wavelengths of light can be measured with an instrument called a **spectrophotometer**. This machine directs beams of light of different wavelengths through a solution of the pigment and measures the fraction of the light transmitted at each wavelength. A graph plotting a pigment's light absorption versus wavelength is called an **absorption spectrum** (**Figure 11.9**).

The absorption spectra of chloroplast pigments provide clues to the relative effectiveness of different wavelengths for driving photosynthesis, since light can perform work in chloroplasts only if it is absorbed. **Figure 11.10a** shows the absorption spectra of three types of pigments in chloroplasts: **chlorophyll a**, the key light-capturing pigment that participates directly in the light reactions; the accessory pigment **chlorophyll b**; and a separate group of accessory pigments called carotenoids. The spectrum of chlorophyll *a* suggests that violet-blue and red light work best for photosynthesis, since they are absorbed, while green is the least effective color. This is confirmed by an **action spectrum** for

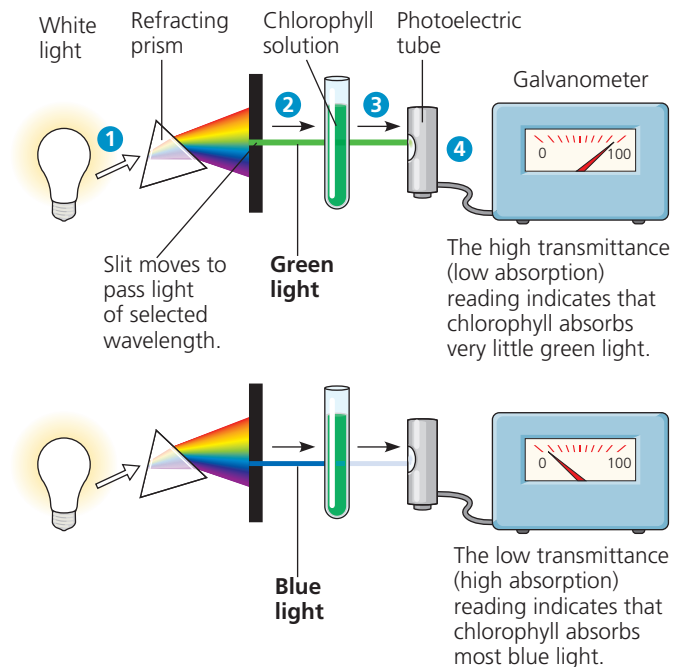
▼ **Figure 11.9**

Research Method Determining an Absorption Spectrum

Application An absorption spectrum is a visual representation of how well a particular pigment absorbs different wavelengths of visible light. Absorption spectra of various chloroplast pigments help scientists decipher the role of each pigment in a plant.

Technique A spectrophotometer measures the relative amounts of light of different wavelengths absorbed and transmitted by a pigment solution.

- 1 White light is separated into colors (wavelengths) by a prism.
- 2 One by one, the different colors of light are passed through the sample (chlorophyll in this example). Green light and blue light are shown here.
- 3 The transmitted light strikes a photoelectric tube, which converts the light energy to electricity.
- 4 The electric current is measured by a galvanometer. The meter indicates the fraction of light transmitted through the sample, from which we can determine the amount of light absorbed.



Results See Figure 11.10a for absorption spectra of three types of chloroplast pigments.

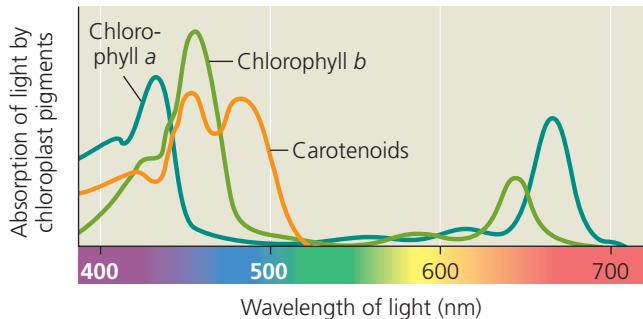
photosynthesis (**Figure 11.10b**), which profiles the relative effectiveness of different wavelengths of radiation in driving the process. An action spectrum is prepared by illuminating chloroplasts with light of different colors and then plotting wavelength against some measure of photosynthetic rate, such as CO_2 consumption or O_2 release. The action spectrum for photosynthesis was first demonstrated by Theodor W. Engelmann, a German botanist, in 1883. Before equipment for measuring O_2 levels had even been invented, Engelmann

▼ Figure 11.10

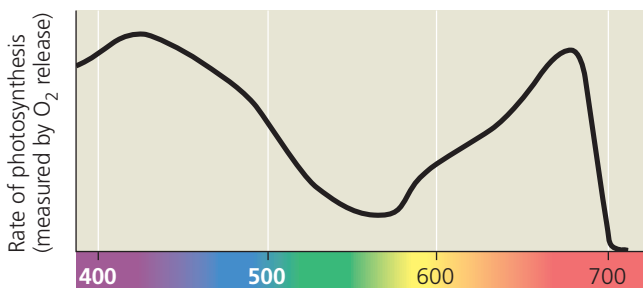
Inquiry Which wavelengths of light are most effective in driving photosynthesis?

Experiment Absorption and action spectra, along with a classic experiment by Theodor W. Engelmann, reveal which wavelengths of light are photosynthetically important.

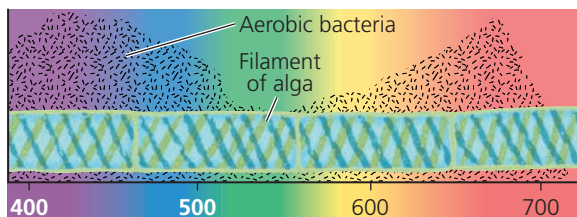
Results



(a) Absorption spectra. The three curves show the wavelengths of light best absorbed by three types of chloroplast pigments.



(b) Action spectrum. This graph plots the rate of photosynthesis versus wavelength. The resulting action spectrum resembles the absorption spectrum for chlorophyll *a* but does not match exactly (see part a). This is partly due to the absorption of light by accessory pigments such as chlorophyll *b* and carotenoids.



(c) Engelmann's experiment. In 1883, Theodor W. Engelmann illuminated a filamentous alga with light that had been passed through a prism, exposing different segments of the alga to different wavelengths. He used aerobic bacteria, which congregate near an oxygen source, to determine which segments of the alga were releasing the most O_2 and thus photosynthesizing most. Bacteria congregated in greatest numbers around the parts of the alga illuminated with violet-blue or red light.

Conclusion The action spectra, confirmed by Engelmann's experiment, show which portions of the spectrum are most effective in driving photosynthesis.

Data from T. W. Engelmann, *Bacterium photometricum*. Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbenseins, *Archiv. für Physiologie* 30:95–124 (1883).

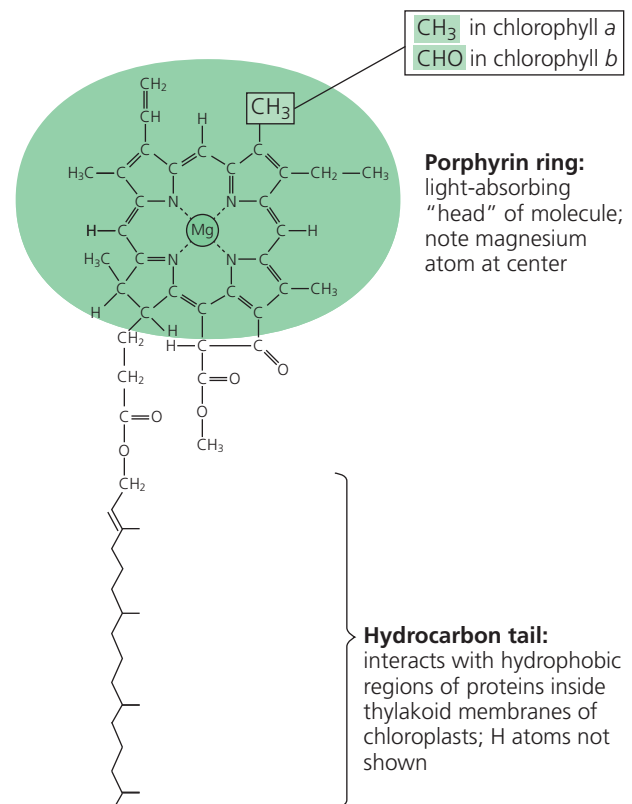
Instructors: A related Experimental Inquiry Tutorial can be assigned in MasteringBiology.

INTERPRET THE DATA ▶ According to the graph, which wavelengths of light drive the highest rates of photosynthesis?

performed a clever experiment in which he used bacteria to measure rates of photosynthesis in filamentous algae (**Figure 11.10c**). His results are a striking match to the modern action spectrum shown in Figure 11.10b.

Notice by comparing Figures 11.10a and 11.10b that the action spectrum for photosynthesis is much broader than the absorption spectrum of chlorophyll *a*. The absorption spectrum of chlorophyll *a* alone underestimates the effectiveness of certain wavelengths in driving photosynthesis. This is partly because accessory pigments with different absorption spectra also present in chloroplasts—including chlorophyll *b* and carotenoids—broaden the spectrum of colors that can be used for photosynthesis. **Figure 11.11** shows the structure of chlorophyll *a* compared with that of chlorophyll *b*. A slight structural difference between them is enough to cause the two pigments to absorb at slightly different wavelengths in the red and blue parts of the spectrum (see Figure 11.10a). As a result, chlorophyll *a* appears blue green and chlorophyll *b* olive green under visible light.

Other accessory pigments include **carotenoids**, hydrocarbons that are various shades of yellow and orange because they absorb violet and blue-green light (see Figure 11.10a). Carotenoids may broaden the spectrum of colors that can



▲ **Figure 11.11 Structure of chlorophyll molecules in chloroplasts of plants.** Chlorophyll *a* and chlorophyll *b* differ in only one of the functional groups bonded to the porphyrin ring. (Also see the space-filling model of chlorophyll in Figure 1.3.)

Animation: Space-Filling Model of Chlorophyll

drive photosynthesis. However, a more important function of at least some carotenoids seems to be *photoprotection*: These compounds absorb and dissipate excessive light energy that would otherwise damage chlorophyll or interact with oxygen, forming reactive oxidative molecules that are dangerous to the cell. Interestingly, carotenoids similar to the photoprotective ones in chloroplasts have a photoprotective role in the human eye. (Carrots, known for aiding night vision, are rich in carotenoids.) These and related molecules are, of course, found naturally in many vegetables and fruits. They are also often advertised in health food products as “phytochemicals” (from the Greek *phyton*, plant), some of which have antioxidant properties. Plants can synthesize all the antioxidants they require, but humans and other animals must obtain some of them from their diets.

Excitation of Chlorophyll by Light

What exactly happens when chlorophyll and other pigments absorb light? The colors corresponding to the absorbed wavelengths disappear from the spectrum of the transmitted and reflected light, but energy cannot disappear. When a molecule absorbs a photon of light, one of the molecule’s electrons is elevated to an orbital where it has more potential energy (see Figure 2.6b). When the electron is in its normal orbital, the pigment molecule is said to be in its ground state. Absorption of a photon boosts an electron to an orbital of higher energy, and the pigment molecule is then said to be in an excited state. The only photons absorbed are those whose energy is exactly equal to the energy difference between the ground state and an excited state, and this energy difference varies from one kind of molecule to another. Thus, a particular compound absorbs only photons corresponding to specific

wavelengths, which is why each pigment has a unique absorption spectrum.

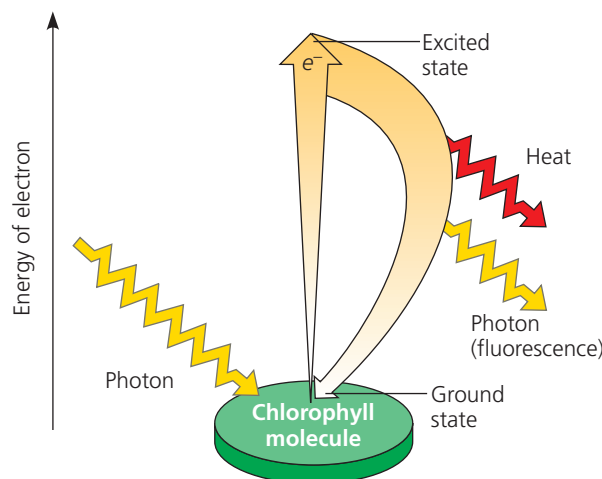
Once absorption of a photon raises an electron to an excited state, the electron cannot stay there long. The excited state, like all high-energy states, is unstable. Generally, when isolated pigment molecules absorb light, their excited electrons drop back down to the ground-state orbital in a billionth of a second, releasing their excess energy as heat. This conversion of light energy to heat is what makes the top of an automobile so hot on a sunny day. (White cars are coolest because their paint reflects all wavelengths of visible light.) In isolation, some pigments, including chlorophyll, emit light as well as heat after absorbing photons. As excited electrons fall back to the ground state, photons are given off, an afterglow called fluorescence. An illuminated solution of chlorophyll isolated from chloroplasts will fluoresce in the red part of the spectrum and also give off heat (Figure 11.12). This is best seen by illuminating with ultraviolet light, which chlorophyll can also absorb (see Figures 11.7 and 11.10a). Viewed under visible light, the fluorescence would be difficult to see against the green of the solution.

A Photosystem: A Reaction-Center Complex Associated with Light-Harvesting Complexes

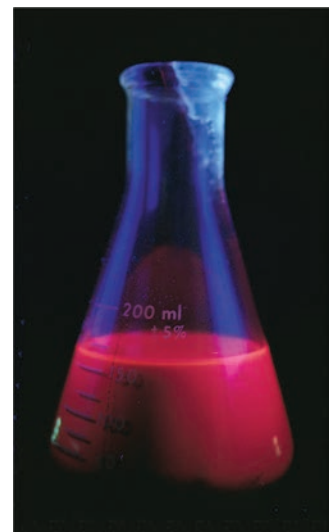
Chlorophyll molecules excited by the absorption of light energy produce very different results in an intact chloroplast than they do in isolation (see Figure 11.12). In their native environment of the thylakoid membrane, chlorophyll molecules are organized along with other small organic molecules and proteins into complexes called photosystems.

► **Figure 11.12 Excitation of isolated chlorophyll by light.** (a) Absorption of a photon causes a transition of the chlorophyll molecule from its ground state to its excited state. The photon boosts an electron to an orbital where it has more potential energy. If the illuminated molecule exists in isolation, its excited electron immediately drops back down to the ground-state orbital, and its excess energy is given off as heat and fluorescence (light). (b) A chlorophyll solution excited with ultraviolet light fluoresces with a red-orange glow.

WHAT IF? ► If a leaf containing the same concentration of chlorophyll as in the solution was exposed to the same ultraviolet light, no fluorescence would be seen. Propose an explanation for the difference in fluorescence emission between the solution and the leaf.



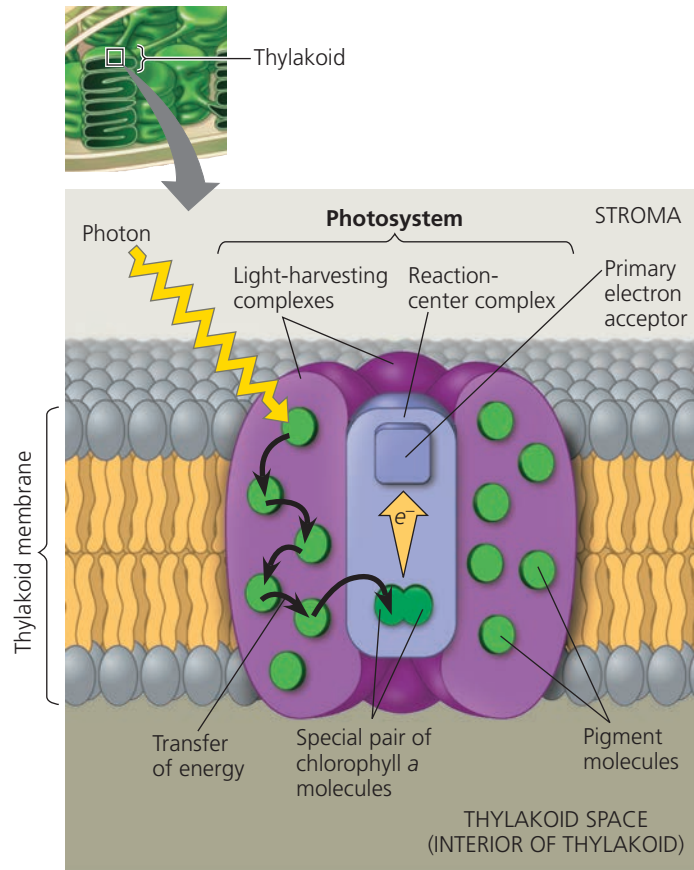
(a) Excitation of isolated chlorophyll molecule



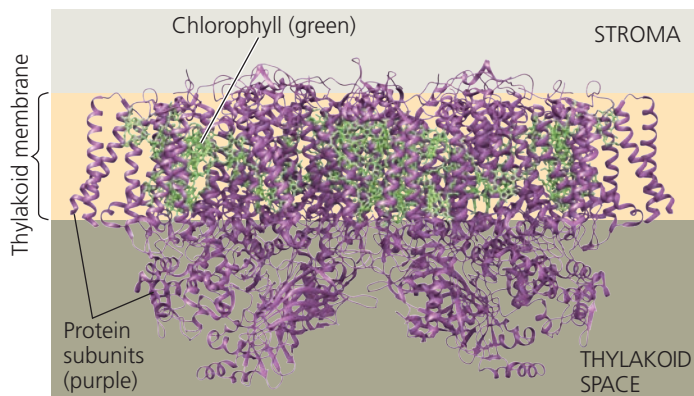
(b) Fluorescence

A **photosystem** is composed of a reaction-center complex surrounded by several light-harvesting complexes (Figure 11.13). The **reaction-center complex** is an

▼ **Figure 11.13 The structure and function of a photosystem.**



(a) **How a photosystem harvests light.** When a photon strikes a pigment molecule in a light-harvesting complex, the energy is passed from molecule to molecule until it reaches the reaction-center complex. Here, an excited electron from the special pair of chlorophyll *a* molecules is transferred to the primary electron acceptor.



(b) **Structure of a photosystem.** This computer model, based on X-ray crystallography, shows two photosystem complexes side by side. Chlorophyll molecules (bright green ball-and-stick models within the membrane; the tails are not shown) are interspersed with protein subunits (purple ribbons; notice the many α helices spanning the membrane). For simplicity, a photosystem will be shown as a single complex in the rest of the chapter.

organized association of proteins holding a special pair of chlorophyll *a* molecules and a primary electron acceptor. Each **light-harvesting complex** consists of various pigment molecules (which may include chlorophyll *a*, chlorophyll *b*, and multiple carotenoids) bound to proteins. The number and variety of pigment molecules enable a photosystem to harvest light over a larger surface area and a larger portion of the spectrum than could any single pigment molecule alone. Together, these light-harvesting complexes act as an antenna for the reaction-center complex. When a pigment molecule absorbs a photon, the energy is transferred from pigment molecule to pigment molecule within a light-harvesting complex, like a human “wave” at a sports arena, until it is passed to the pair of chlorophyll *a* molecules in the reaction-center complex. The pair of chlorophyll *a* molecules in the reaction-center complex are special because their molecular environment—their location and the other molecules with which they are associated—enables them to use the energy from light not only to boost one of their electrons to a higher energy level, but also to transfer it to a different molecule—the **primary electron acceptor**, which is a molecule capable of accepting electrons and becoming reduced.

The solar-powered transfer of an electron from the reaction-center chlorophyll *a* pair to the primary electron acceptor is the first step of the light reactions. As soon as the chlorophyll electron is excited to a higher energy level, the primary electron acceptor captures it; this is a redox reaction. In the flask shown in Figure 11.12b, isolated chlorophyll fluoresces because there is no electron acceptor, so electrons of photoexcited chlorophyll drop right back to the ground state. In the structured environment of a chloroplast, however, an electron acceptor is readily available, and the potential energy represented by the excited electron is not dissipated as light and heat. Thus, each photosystem—a reaction-center complex surrounded by light-harvesting complexes—functions in the chloroplast as a unit. It converts light energy to chemical energy, which will ultimately be used for the synthesis of sugar.

The thylakoid membrane is populated by two types of photosystems that cooperate in the light reactions of photosynthesis: **photosystem II (PS II)** and **photosystem I (PS I)**. (They were named in order of their discovery, but photosystem II functions first in the light reactions.) Each has a characteristic reaction-center complex—a particular kind of primary electron acceptor next to a special pair of chlorophyll *a* molecules associated with specific proteins. The reaction-center chlorophyll *a* of photosystem II is known as P680 because this pigment is best at absorbing light having a wavelength of 680 nm (in the red part of the spectrum). The chlorophyll *a* at the reaction-center complex of photosystem I is called P700 because it most effectively absorbs light of wavelength 700 nm (in the far-red part of the spectrum). These two pigments, P680 and P700, are nearly identical chlorophyll *a* molecules. However, their association with different proteins in the thylakoid membrane affects the

electron distribution in the two pigments and accounts for the slight differences in their light-absorbing properties. Now let's see how the two types of photosystems work together in using light energy to generate ATP and NADPH, the two main products of the light reactions.

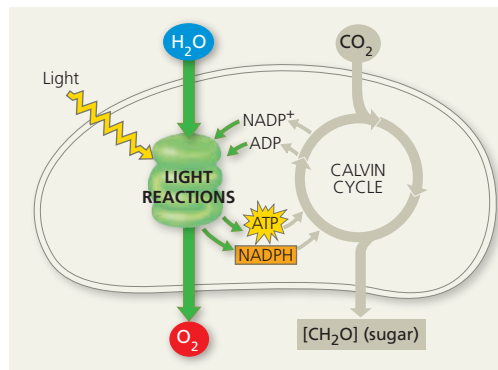
Linear Electron Flow

Light drives the synthesis of ATP and NADPH by energizing the two types of photosystems embedded in the thylakoid membranes of chloroplasts. The key to this energy transformation is a flow of electrons through the photosystems and other molecular components built into the thylakoid membrane. This is called **linear electron flow**, and it occurs during the light reactions of photosynthesis, as shown in **Figure 11.14**. The numbered steps in the text correspond to the numbered steps in the figure.

- 1 A photon of light strikes one of the pigment molecules in a light-harvesting complex of PS II, boosting one of its electrons to a higher energy level. As this electron falls

back to its ground state, an electron in a nearby pigment molecule is simultaneously raised to an excited state. The process continues, with the energy being relayed to other pigment molecules until it reaches the P680 pair of chlorophyll *a* molecules in the PS II reaction-center complex. It excites an electron in this pair of chlorophylls to a higher energy state.

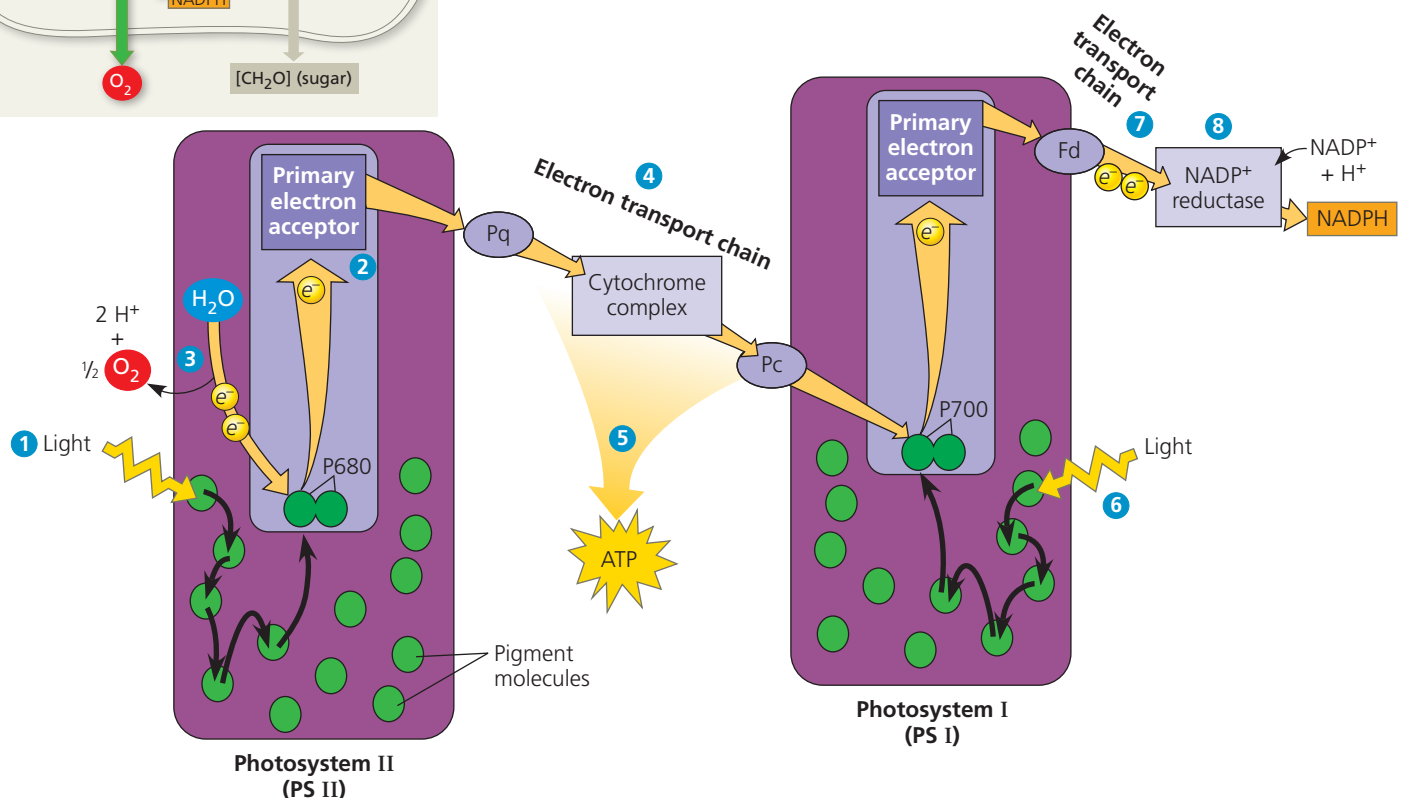
- 2 This electron is transferred from the excited P680 to the primary electron acceptor. We can refer to the resulting form of P680, missing the negative charge of an electron, as $P680^+$.
- 3 An enzyme catalyzes the splitting of a water molecule into two electrons, two hydrogen ions (H^+), and an oxygen atom. The electrons are supplied one by one to the $P680^+$ pair, each electron replacing one transferred to the primary electron acceptor. ($P680^+$ is the strongest biological oxidizing agent known; its electron "hole" must be filled. This greatly facilitates the transfer of electrons from the split water molecule.) The H^+ are released into the thylakoid space (interior of the thylakoid). The oxygen atom



✓ **Figure 11.14** How linear electron flow during the light reactions generates ATP and NADPH. The gold arrows trace the flow of light-driven electrons from water to NADPH. The black arrows trace the transfer of energy from pigment molecule to pigment molecule.



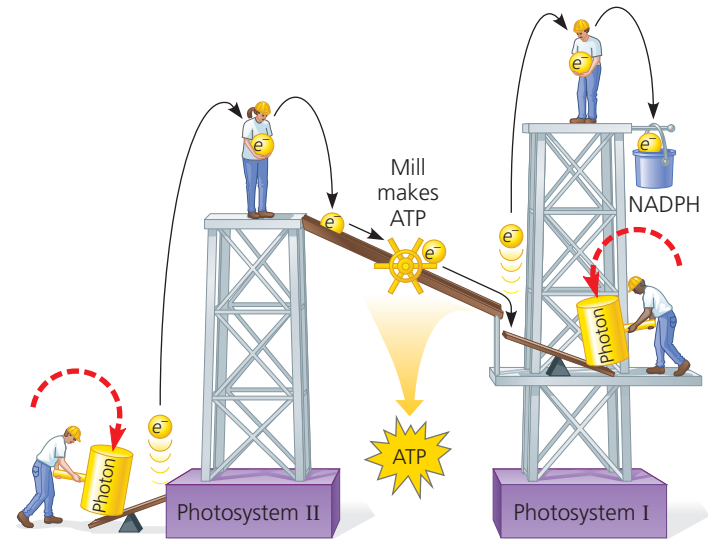
Figure Walkthrough



immediately combines with an oxygen atom generated by the splitting of another water molecule, forming O_2 .

- 4 Each photoexcited electron passes from the primary electron acceptor of PS II to PS I via an electron transport chain, the components of which are similar to those of the electron transport chain that functions in cellular respiration. The electron transport chain between PS II and PS I is made up of the electron carrier plastoquinone (Pq), a cytochrome complex, and a protein called plastocyanin (Pc). Each component carries out redox reactions as electrons flow down the electron transport chain, releasing free energy that is used to pump protons (H^+) into the thylakoid space, contributing to a proton gradient across the thylakoid membrane.
- 5 The potential energy stored in the proton gradient is used to make ATP in a process called chemiosmosis, to be discussed shortly.
- 6 Meanwhile, light energy has been transferred via light-harvesting complex pigments to the PS I reaction-center complex, exciting an electron of the P700 pair of chlorophyll *a* molecules located there. The photoexcited electron is then transferred to PS I's primary electron acceptor, creating an electron "hole" in the P700—which we now can call $P700^+$. In other words, $P700^+$ can now act as an electron acceptor, accepting an electron that reaches the bottom of the electron transport chain from PS II.
- 7 Photoexcited electrons are passed in a series of redox reactions from the primary electron acceptor of PS I down a second electron transport chain through the protein ferredoxin (Fd). (This chain does not create a proton gradient and thus does not produce ATP.)
- 8 The enzyme NADP⁺ reductase catalyzes the transfer of electrons from Fd to NADP⁺. Two electrons are required for its reduction to NADPH. Electrons in NADPH are at a higher energy level than they are in water (where they started), so they are more readily available for the

▼ **Figure 11.15** A mechanical analogy for linear electron flow during the light reactions.

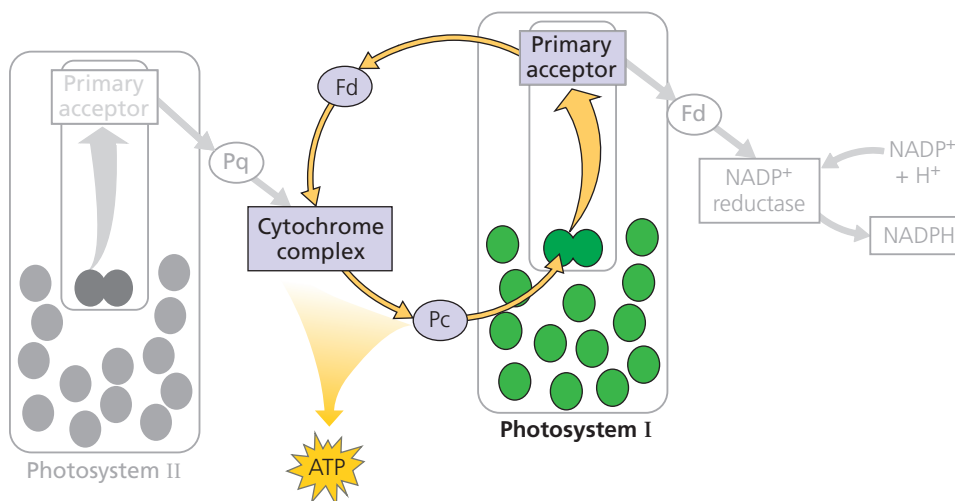


reactions of the Calvin cycle. This process also removes an H^+ from the stroma.

The energy changes of electrons during their linear flow through the light reactions are shown in a mechanical analogy in **Figure 11.15**. Although the scheme shown in Figures 11.14 and 11.15 may seem complicated, do not lose track of the big picture: The light reactions use solar power to generate ATP and NADPH, which provide chemical energy and reducing power, respectively, to the carbohydrate-synthesizing reactions of the Calvin cycle.

Cyclic Electron Flow

In certain cases, photoexcited electrons can take an alternative path called **cyclic electron flow**, which uses photosystem I but not photosystem II. You can see in **Figure 11.16** that cyclic flow is a short circuit: The electrons cycle back from ferredoxin (Fd) to the cytochrome complex, then



◀ **Figure 11.16** Cyclic electron flow.

Photoexcited electrons from PS I are occasionally shunted back from ferredoxin (Fd) to chlorophyll via the cytochrome complex and plastocyanin (Pc). This electron shunt supplements the supply of ATP (via chemiosmosis) but produces no NADPH. The "shadow" of linear electron flow is included in the diagram for comparison with the cyclic route. The two Fd molecules in this diagram are actually one and the same—the final electron carrier in the electron transport chain of PS I—although it is depicted twice to clearly show its role in two parts of the process.

VISUAL SKILLS ▶ Look at Figure 11.15 and explain how you would alter it to show a mechanical analogy for cyclic electron flow.

via a plastocyanin molecule (Pc) to a P700 chlorophyll in the PS I reaction-center complex. There is no production of NADPH and no release of oxygen that results from this process. On the other hand, cyclic flow does generate ATP.

Rather than having both PS II and PS I, several of the currently existing groups of photosynthetic bacteria are known to have a single photosystem related to either PS II or PS I. For these species, which include the purple sulfur bacteria (see Figure 11.2e) and the green sulfur bacteria, cyclic electron flow is the one and only means of generating ATP during the process of photosynthesis. Evolutionary biologists hypothesize that these bacterial groups are descendants of ancestral bacteria in which photosynthesis first evolved, in a form similar to cyclic electron flow.

Cyclic electron flow can also occur in photosynthetic species that possess both photosystems; this includes some prokaryotes, such as the cyanobacteria shown in Figure 11.2d, as well as the eukaryotic photosynthetic species that have been tested thus far. Although the process is probably in part an “evolutionary leftover,” research suggests it plays at least one beneficial role for these organisms. Plants with mutations that render them unable to carry out cyclic electron flow are capable of growing well in low light, but do not grow well where light is intense. This is evidence for the idea that cyclic electron flow may be photoprotective. Later you’ll learn more about cyclic electron flow as it relates to a particular adaptation of photosynthesis (C_4 plants; see Concept 11.4).

Whether ATP synthesis is driven by linear or cyclic electron flow, the actual mechanism is the same. Before we move on to consider the Calvin cycle, let’s review chemiosmosis, the process that uses membranes to couple redox reactions to ATP production.

A Comparison of Chemiosmosis in Chloroplasts and Mitochondria

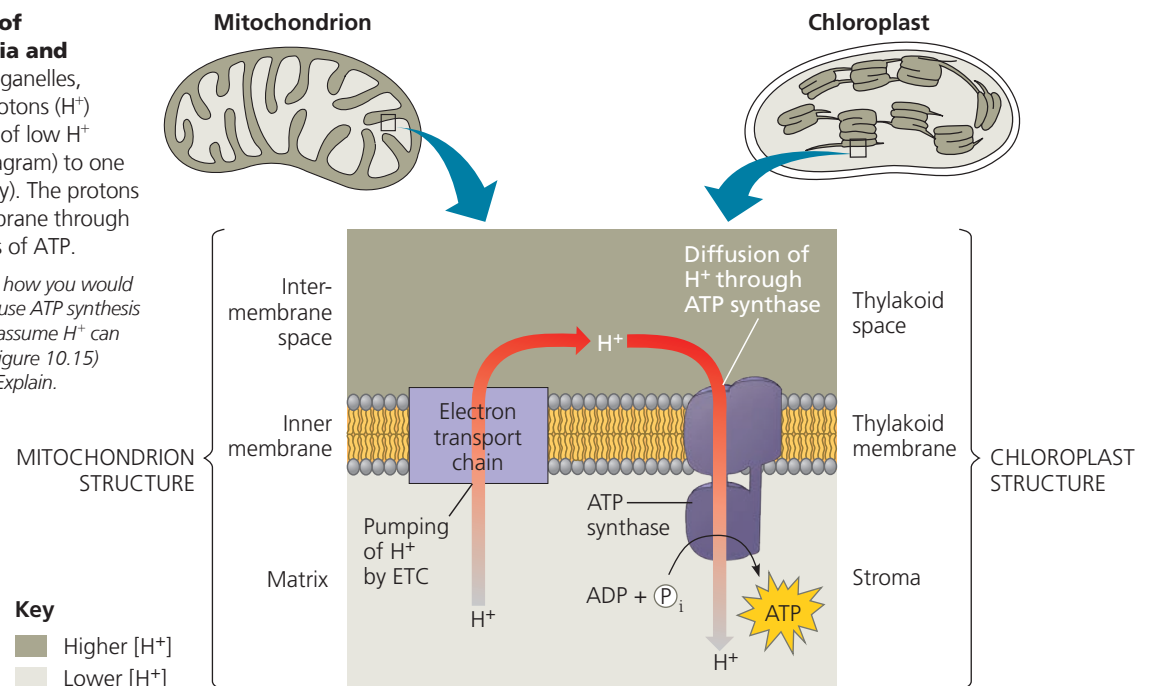
Chloroplasts and mitochondria generate ATP by the same basic mechanism: chemiosmosis (see Figure 10.15). An electron transport chain pumps protons (H^+) across a membrane as electrons are passed through a series of carriers that are progressively more electronegative. Thus, electron transport chains transform redox energy to a proton-motive force, potential energy stored in the form of an H^+ gradient across a membrane. An ATP synthase complex in the same membrane couples the diffusion of hydrogen ions down their gradient to the phosphorylation of ADP, forming ATP.

Some of the electron carriers, including the iron-containing proteins called cytochromes, are very similar in chloroplasts and mitochondria. The ATP synthase complexes of the two organelles are also quite similar. But there are noteworthy differences between photophosphorylation in chloroplasts and oxidative phosphorylation in mitochondria. Both work by way of chemiosmosis, but in chloroplasts, the high-energy electrons dropped down the transport chain come from water, while in mitochondria, they are extracted from organic molecules (which are thus oxidized). Chloroplasts do not need molecules from food to make ATP; their photosystems capture light energy and use it to drive the electrons from water to the top of the transport chain. In other words, mitochondria use chemiosmosis to transfer chemical energy from food molecules to ATP, whereas chloroplasts use it to transform light energy into chemical energy in ATP.

Although the spatial organization of chemiosmosis differs slightly between chloroplasts and mitochondria, it is easy to see similarities in the two (Figure 11.17). Electron transport chain

➤ Figure 11.17 Comparison of chemiosmosis in mitochondria and chloroplasts. In both kinds of organelles, electron transport chains pump protons (H^+) across a membrane from a region of low H^+ concentration (light gray in this diagram) to one of high H^+ concentration (dark gray). The protons then diffuse back across the membrane through ATP synthase, driving the synthesis of ATP.

MAKE CONNECTIONS ➤ Describe how you would change the pH in order to artificially cause ATP synthesis (a) outside an isolated mitochondrion (assume H^+ can freely cross the outer membrane; see Figure 10.15) and (b) in the stroma of a chloroplast. Explain.



proteins in the inner membrane of the mitochondrion pump protons from the mitochondrial matrix out to the intermembrane space, which then serves as a reservoir of hydrogen ions. Similarly, electron transport chain proteins in the thylakoid membrane of the chloroplast pump protons from the stroma into the thylakoid space (interior of the thylakoid), which functions as the H^+ reservoir. If you imagine the cristae of mitochondria pinching off from the inner membrane, this may help you see how the thylakoid space and the intermembrane space are

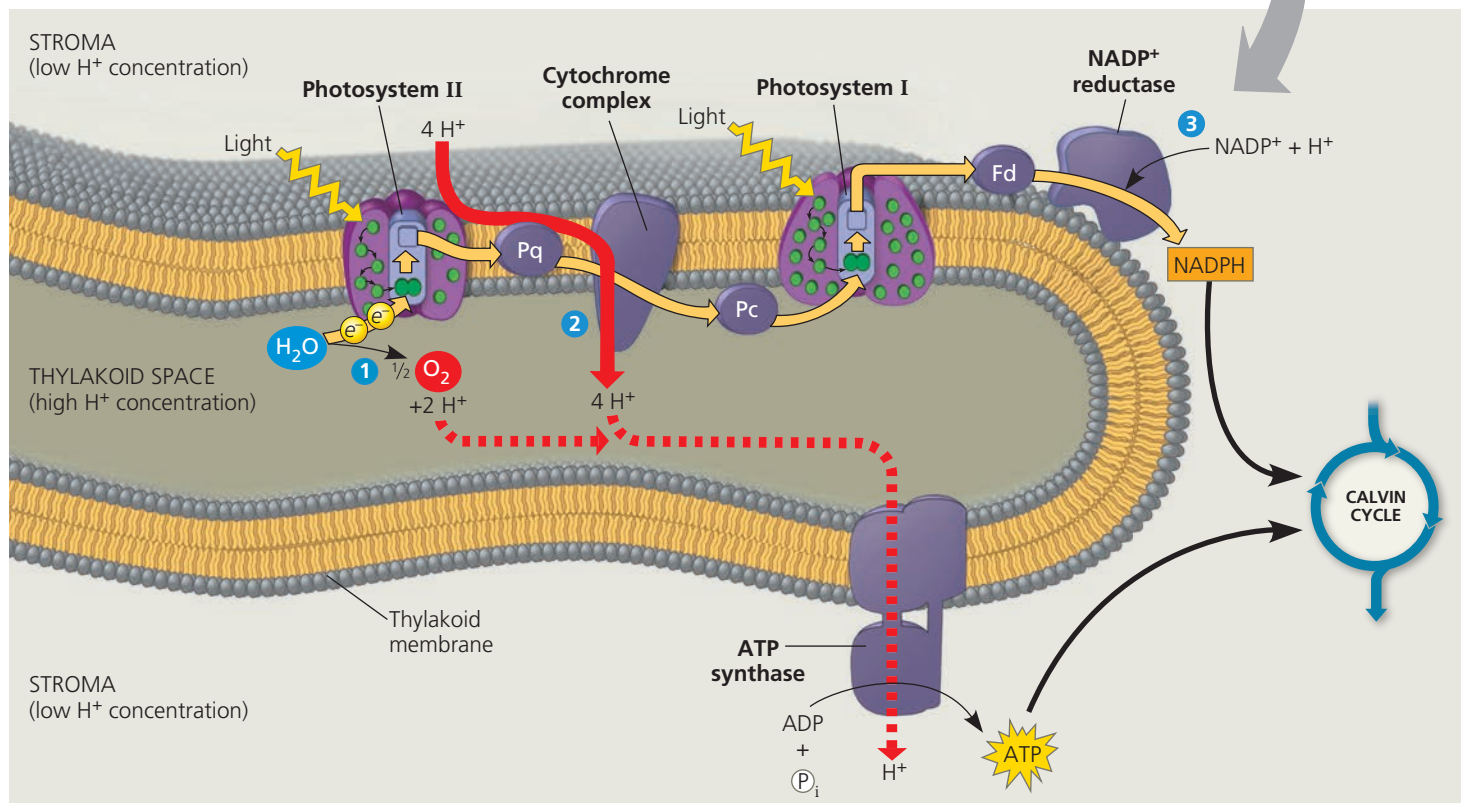
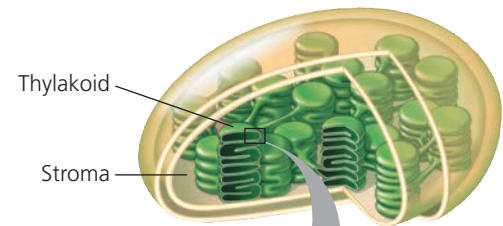
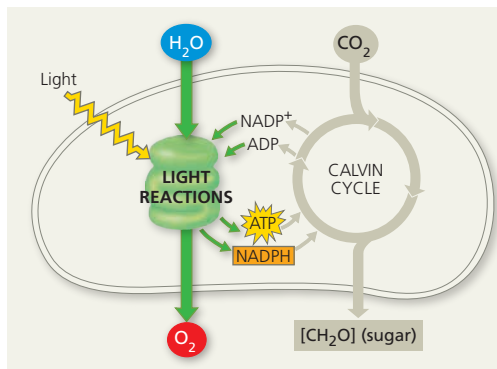
comparable spaces in the two organelles, while the mitochondrial matrix is analogous to the stroma of the chloroplast.

In the mitochondrion, protons diffuse down their concentration gradient from the intermembrane space through ATP synthase to the matrix, driving ATP synthesis. In the chloroplast, ATP is synthesized as the hydrogen ions diffuse from the thylakoid space back to the stroma through ATP synthase complexes (Figure 11.18), whose catalytic knobs are on the stroma side of the membrane. Thus, ATP forms in the

▼ Figure 11.18 The light reactions and chemiosmosis: Current model of the organization of the thylakoid membrane. The gold arrows track the linear electron flow outlined in Figure 11.14. At least three steps in the light reactions contribute to the H^+ gradient across the thylakoid membrane: **1** Water is split by photosystem II

on the side of the membrane facing the thylakoid space; **2** as plastoquinone (Pq) transfers electrons to the cytochrome complex, four protons are translocated across the membrane into the thylakoid space; and **3** a hydrogen ion is removed from the stroma when it is taken up by $NADP^+$. Notice that in step 2, hydrogen ions are being

pumped from the stroma into the thylakoid space, as in Figure 11.17. The diffusion of H^+ from the thylakoid space back to the stroma (along the H^+ concentration gradient) powers the ATP synthase. These light-driven reactions store chemical energy in NADPH and ATP, which shuttle the energy to the carbohydrate-producing Calvin cycle.



stroma, where it is used to help drive sugar synthesis during the Calvin cycle.

The proton (H^+) gradient, or pH gradient, across the thylakoid membrane is substantial. When chloroplasts in an experimental setting are illuminated, the pH in the thylakoid space drops to about 5 (the H^+ concentration increases), and the pH in the stroma increases to about 8 (the H^+ concentration decreases). This gradient of three pH units corresponds to a thousandfold difference in H^+ concentration. If the lights are then turned off, the pH gradient is abolished, but it can quickly be restored by turning the lights back on. Experiments such as this provided strong evidence in support of the chemiosmotic model.

The currently accepted model for the organization of the light-reaction “machinery” within the thylakoid membrane is based on several research studies. Each of the molecules and molecular complexes in the figure is present in numerous copies in each thylakoid. Notice that NADPH, like ATP, is produced on the side of the membrane facing the stroma, where the Calvin cycle reactions take place.

Let’s summarize the light reactions. Electron flow pushes electrons from water, where they are at a state of low potential energy, ultimately to NADPH, where they are stored at a state of high potential energy. The light-driven electron flow also generates ATP. Thus, the equipment of the thylakoid membrane converts light energy to chemical energy stored in ATP and NADPH. Oxygen is produced as a by-product. Let’s now see how the Calvin cycle uses the products of the light reactions to synthesize sugar from CO_2 .



BioFlix® Animation: The Light Reactions

CONCEPT CHECK 11.2

1. What color of light is *least* effective in driving photosynthesis? Explain.
2. In the light reactions, what is the initial electron donor? Where do the electrons finally end up?
3. **WHAT IF? >** In an experiment, isolated chloroplasts placed in an illuminated solution with the appropriate chemicals can carry out ATP synthesis. Predict what would happen to the rate of synthesis if a compound is added to the solution that makes membranes freely permeable to hydrogen ions.

For suggested answers, see Appendix A.

CONCEPT 11.3

The Calvin cycle uses the chemical energy of ATP and NADPH to reduce CO_2 to sugar

The Calvin cycle is similar to the citric acid cycle in that a starting material is regenerated after some molecules enter

and others exit the cycle. However, the citric acid cycle is catabolic, oxidizing acetyl CoA and using the energy to synthesize ATP, while the Calvin cycle is anabolic, building carbohydrates from smaller molecules and consuming energy. Carbon enters the Calvin cycle in the form of CO_2 and leaves in the form of sugar. The cycle spends ATP as an energy source and consumes NADPH as reducing power for adding high-energy electrons to make the sugar.

As we mentioned in Concept 11.1, the carbohydrate produced directly from the Calvin cycle is not glucose. It is actually a three-carbon sugar called **glyceraldehyde 3-phosphate (G3P)**. For the net synthesis of *one* molecule of G3P, the cycle must take place three times, fixing *three* molecules of CO_2 —one per turn of the cycle. (Recall that the term *carbon fixation* refers to the initial incorporation of CO_2 into organic material.) As we trace the steps of the Calvin cycle, keep in mind that we are following three molecules of CO_2 through the reactions. **Figure 11.19** divides the Calvin cycle into three phases: carbon fixation, reduction, and regeneration of the CO_2 acceptor.

Phase 1: Carbon fixation. The Calvin cycle incorporates each CO_2 molecule, one at a time, by attaching it to a five-carbon sugar named ribulose biphosphate (abbreviated RuBP). The enzyme that catalyzes this first step is RuBP carboxylase-oxygenase, or **rubisco**. (This is the most abundant protein in chloroplasts and is also thought to be the most abundant protein on Earth.) The product of the reaction is a six-carbon intermediate that is short-lived because it is so energetically unstable that it immediately splits in half, forming two molecules of 3-phosphoglycerate (for each CO_2 fixed).

Phase 2: Reduction. Each molecule of 3-phosphoglycerate receives an additional phosphate group from ATP, becoming 1,3-bisphosphoglycerate. Next, a pair of electrons donated from NADPH reduces 1,3-bisphosphoglycerate, which also loses a phosphate group in the process, becoming glyceraldehyde 3-phosphate (G3P). Specifically, the electrons from NADPH reduce a carboxyl group on 1,3-bisphosphoglycerate to the aldehyde group of G3P, which stores more potential energy. G3P is a sugar—the same three-carbon sugar formed in glycolysis by the splitting of glucose (see Figure 10.9). Notice in Figure 11.19 that for every *three* molecules of CO_2 that enter the cycle, there are *six* molecules of G3P formed. But only one molecule of this three-carbon sugar can be counted as a net gain of carbohydrate because the rest are required to complete the cycle. The cycle began with 15 carbons’ worth of carbohydrate in the form of three molecules of the five-carbon sugar RuBP. Now there are 18 carbons’ worth of carbohydrate in the form of six molecules of G3P. One molecule exits the cycle to be used by the plant cell, but the other five molecules must be recycled to regenerate the three molecules of RuBP.

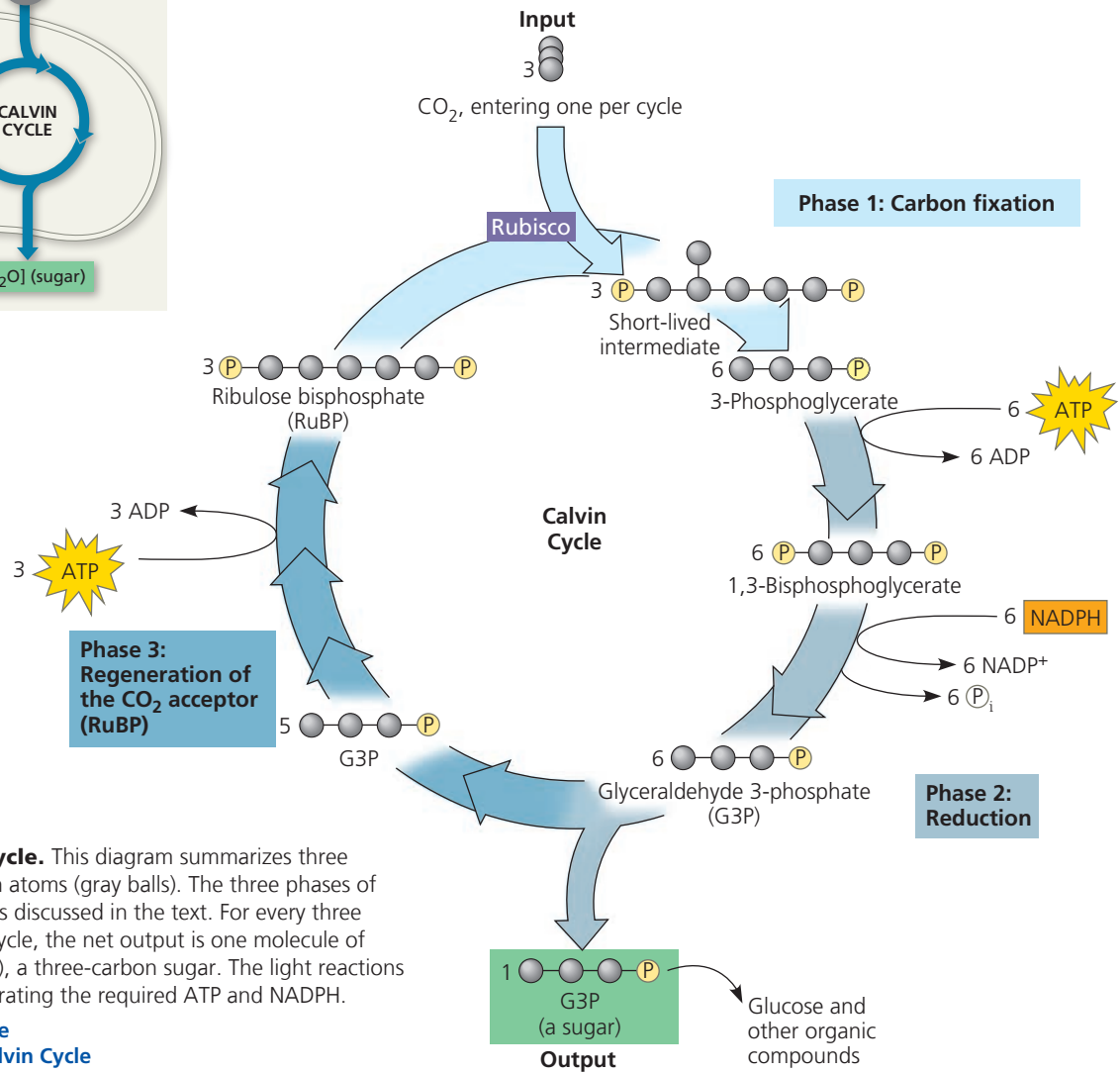
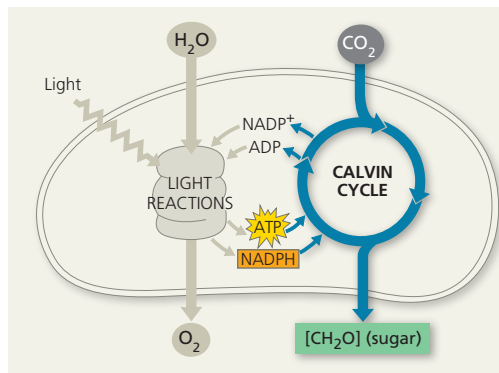


Figure 11.19 The Calvin cycle. This diagram summarizes three turns of the cycle, tracking carbon atoms (gray balls). The three phases of the cycle correspond to the phases discussed in the text. For every three molecules of CO₂ that enter the cycle, the net output is one molecule of glyceraldehyde 3-phosphate (G3P), a three-carbon sugar. The light reactions sustain the Calvin cycle by regenerating the required ATP and NADPH.

Animation: The Calvin Cycle
BioFlix® Animation: The Calvin Cycle

Phase 3: Regeneration of the CO₂ acceptor (RuBP).

In a complex series of reactions, the carbon skeletons of five molecules of G3P are rearranged by the last steps of the Calvin cycle into three molecules of RuBP. To accomplish this, the cycle spends three more molecules of ATP. The RuBP is now prepared to receive CO₂ again, and the cycle continues.

For the net synthesis of one G3P molecule, the Calvin cycle consumes a total of nine molecules of ATP and six molecules of NADPH. The light reactions regenerate the ATP and NADPH. The G3P spun off from the Calvin cycle becomes the starting material for metabolic pathways that synthesize other organic compounds, including glucose (from two molecules of G3P), the disaccharide sucrose, and other carbohydrates. Neither the light reactions nor the Calvin cycle alone can make sugar from CO₂. Photosynthesis is an emergent property of the intact chloroplast, which integrates the two stages of photosynthesis.

CONCEPT CHECK 11.3

1. To synthesize one glucose molecule, the Calvin cycle uses _____ molecules of CO₂, _____ molecules of ATP, and _____ molecules of NADPH.
2. How are the large numbers of ATP and NADPH molecules used during the Calvin cycle consistent with the high value of glucose as an energy source?
3. **WHAT IF? >** Explain why a poison that inhibits an enzyme of the Calvin cycle will also inhibit the light reactions.
4. **DRAW IT >** Redraw the cycle in Figure 11.19 using numerals to indicate the numbers of carbons instead of gray balls, multiplying at each step to ensure that you have accounted for all the carbons. In what forms do the carbon atoms enter and leave the cycle?
5. **MAKE CONNECTIONS >** Review Figures 10.9 and 11.19. Discuss the roles of intermediate and product played by glyceraldehyde 3-phosphate (G3P) in the two processes shown in these figures.

For suggested answers, see Appendix A.

CONCEPT 11.4

Alternative mechanisms of carbon fixation have evolved in hot, arid climates

EVOLUTION Ever since plants first moved onto land about 475 million years ago, they have been adapting to the problems of terrestrial life, particularly the problem of dehydration. In Concept 36.4, we will consider anatomical adaptations that help plants conserve water, while in this chapter we are concerned with metabolic adaptations. The solutions often involve trade-offs. An important example is the balance between photosynthesis and the prevention of excessive water loss from the plant. The CO_2 required for photosynthesis enters a leaf (and the resulting O_2 exits) via stomata, the pores on the leaf surface (see Figure 11.4). However, stomata are also the main avenues of transpiration, the evaporative loss of water from leaves. On a hot, dry day, most plants close their stomata, a response that conserves water but also reduces CO_2 levels. With stomata even partially closed, CO_2 concentrations begin to decrease in the air spaces within the leaf, and the concentration of O_2 released from the light reactions begins to increase. These conditions within the leaf favor an apparently wasteful process called photorespiration.

Photorespiration: An Evolutionary Relic?

In most plants, initial fixation of carbon occurs via rubisco, the Calvin cycle enzyme that adds CO_2 to ribulose biphosphate. Such plants are called **C_3 plants** because the first organic product of carbon fixation is a three-carbon compound, 3-phosphoglycerate (see Figure 11.19). Rice, wheat, and soybeans are C_3 plants that are important in agriculture. When their stomata partially close on hot, dry days, C_3 plants produce less sugar because the declining level of CO_2 in the leaf starves the Calvin cycle. In addition, rubisco is capable of binding O_2 in place of CO_2 . As CO_2 becomes scarce within the air spaces of the leaf and O_2 builds up, rubisco adds O_2 to the Calvin cycle instead of CO_2 . The product splits, and a two-carbon compound leaves the chloroplast. Peroxisomes and mitochondria within the plant cell rearrange and split this compound, releasing CO_2 . The process is called **photorespiration** because it occurs in the light (*photo*) and consumes O_2 while producing CO_2 (*respiration*). However, unlike normal cellular respiration, photorespiration uses ATP rather than generating it. And unlike photosynthesis, photorespiration produces no sugar. In fact, photorespiration *decreases* photosynthetic output by siphoning organic material from the Calvin cycle and releasing CO_2 that would otherwise be fixed. This CO_2 can eventually be fixed if it is still in the leaf once the CO_2 concentration builds up to a high enough level. In the meantime, though, the process is energetically costly, much like a hamster running on its wheel.

How can we explain the existence of a metabolic process that seems to be counterproductive for the plant? According to one hypothesis, photorespiration is evolutionary baggage—a metabolic relic from a much earlier time when the atmosphere had less O_2 and more CO_2 than it does today. In the ancient atmosphere that prevailed when rubisco first evolved, the ability of the enzyme's active site to bind O_2 would have made little difference. The hypothesis suggests that modern rubisco retains some of its chance affinity for O_2 , which is now so concentrated in the atmosphere that a certain amount of photorespiration is inevitable. There is also some evidence that photorespiration may provide protection against the damaging products of the light reactions, which build up when the Calvin cycle slows due to low CO_2 .

In many types of plants—including a significant number of crop plants—photorespiration drains away as much as 50% of the carbon fixed by the Calvin cycle. Indeed, if photorespiration could be reduced in certain plant species without otherwise affecting photosynthetic productivity, crop yields and food supplies might increase.

In some plant species, alternate modes of carbon fixation have evolved that minimize photorespiration and optimize the Calvin cycle—even in hot, arid climates. The two most important of these photosynthetic adaptations are C_4 photosynthesis and crassulacean acid metabolism (CAM).

C_4 Plants

The **C_4 plants** are so named because they preface the Calvin cycle with an alternate mode of carbon fixation that forms a four-carbon compound as its first product. The C_4 pathway is believed to have evolved independently at least 45 separate times and is used by several thousand species in at least 19 plant families. Among the C_4 plants important to agriculture are sugarcane and corn, members of the grass family.

The anatomy of a C_4 leaf is correlated with the mechanism of C_4 photosynthesis. In C_4 plants, there are two distinct types of photosynthetic cells: bundle-sheath cells and mesophyll cells. **Bundle-sheath cells** are arranged into tightly packed sheaths around the veins of the leaf (Figure 11.20). Between the bundle sheath and the leaf surface are the more loosely arranged mesophyll cells, which, in C_4 leaves, are closely associated and never more than two to three cells away from the bundle-sheath cells. The Calvin cycle is confined to the chloroplasts of the bundle-sheath cells. However, the Calvin cycle is preceded by incorporation of CO_2 into organic compounds in the mesophyll cells. See the numbered steps in Figure 11.20, which are also described here:

- 1 The first step is carried out by an enzyme present only in mesophyll cells called **PEP carboxylase**. This enzyme adds CO_2 to phosphoenolpyruvate (PEP), forming the four-carbon product oxaloacetate. PEP carboxylase has a much higher affinity for CO_2 than does rubisco and

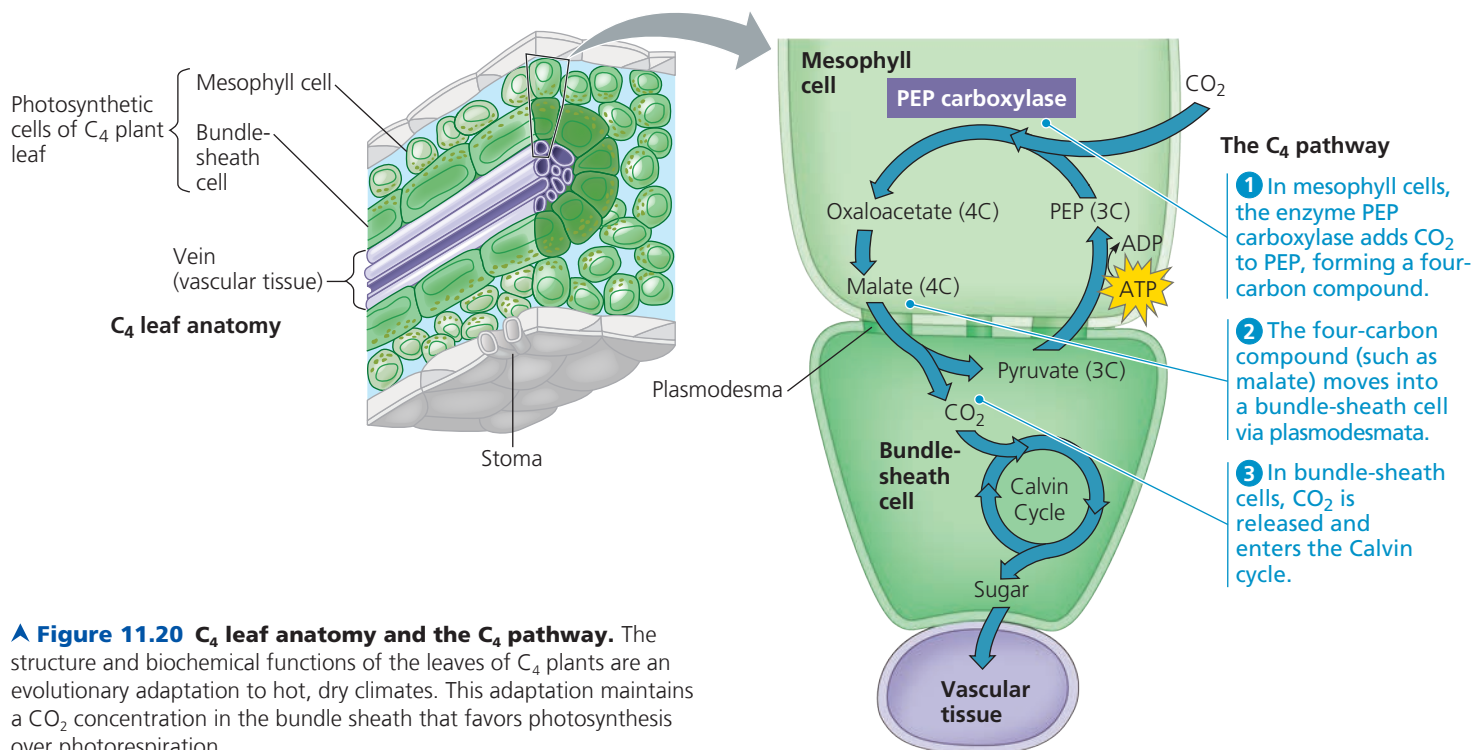


Figure 11.20 C₄ leaf anatomy and the C₄ pathway. The structure and biochemical functions of the leaves of C₄ plants are an evolutionary adaptation to hot, dry climates. This adaptation maintains a CO₂ concentration in the bundle sheath that favors photosynthesis over photorespiration.

no affinity for O₂. Therefore, PEP carboxylase can fix carbon efficiently when rubisco cannot—that is, when it is hot and dry and stomata are partially closed, causing CO₂ concentration in the leaf to be lower and O₂ concentration to be relatively higher.

- 2 After the CO₂ is fixed in the mesophyll cells, the four-carbon products (malate in the example shown in Figure 11.20) are exported to bundle-sheath cells through plasmodesmata (see Figure 7.29).
- 3 Within the bundle-sheath cells, the four-carbon compounds release CO₂, which is re-fixed into organic material by rubisco and the Calvin cycle. The same reaction regenerates pyruvate, which is transported to mesophyll cells. There, ATP is used to convert pyruvate to PEP, which can accept addition of another CO₂, allowing the reaction cycle to continue. This ATP can be thought of, in a sense, as the “price” of concentrating CO₂ in the bundle-sheath cells. To generate this extra ATP, bundle-sheath cells carry out cyclic electron flow, the process described earlier in this chapter (see Figure 11.16). In fact, these cells contain PS I but no PS II, so cyclic electron flow is their only photosynthetic mode of generating ATP.

In effect, the mesophyll cells of a C₄ plant pump CO₂ into the bundle-sheath cells, keeping the CO₂ concentration in those cells high enough for rubisco to bind CO₂ rather than O₂. The cyclic series of reactions involving PEP carboxylase and the regeneration of PEP can be thought of as an ATP-powered pump that concentrates CO₂. In this way, C₄ photosynthesis spends ATP energy to minimize photorespiration and enhance sugar production. This adaptation is especially

advantageous in hot regions with intense sunlight, where stomata partially close during the day, and it is in such environments that C₄ plants evolved and thrive today.

The concentration of CO₂ in the atmosphere has drastically increased since the Industrial Revolution began in the 1800s, and it continues to rise today due to human activities such as the burning of fossil fuels. The resulting global climate change, including an increase in average temperatures around the planet, may have far-reaching effects on plant species. Scientists are concerned that increasing CO₂ concentration and temperature may affect C₃ and C₄ plants differently, thus changing the relative abundance of these species in a given plant community.

Which type of plant would stand to gain more from increasing CO₂ levels? Recall that in C₃ plants, the binding of O₂ rather than CO₂ by rubisco leads to photorespiration, lowering the efficiency of photosynthesis. C₄ plants overcome this problem by concentrating CO₂ in the bundle-sheath cells at the cost of ATP. Rising CO₂ levels should benefit C₃ plants by lowering the amount of photorespiration that occurs. At the same time, rising temperatures have the opposite effect, increasing photorespiration. (Other factors such as water availability may also come into play.) In contrast, many C₄ plants could be largely unaffected by increasing CO₂ levels or temperature. Researchers have investigated aspects of this question in several studies; you can work with data from one such experiment in the **Scientific Skills Exercise**. In different regions, the particular combination of CO₂ concentration and temperature is likely to alter the balance of C₃ and C₄ plants in varying ways. The effects of such a widespread and variable change in community structure are unpredictable and thus a cause of legitimate concern.

SCIENTIFIC SKILLS EXERCISE

Making Scatter Plots with Regression Lines

Does Atmospheric CO₂ Concentration Affect the Productivity of Agricultural Crops? The atmospheric concentration of CO₂ has been rising globally, and scientists wondered whether this would affect C₃ and C₄ plants differently. In this exercise, you will make a scatter plot to examine the relationship between CO₂ concentration and growth of both corn (maize), a C₄ crop plant, and velvetleaf, a C₃ weed found in cornfields.

How the Experiment Was Done Researchers grew corn and velvetleaf plants under controlled conditions for 45 days, where all plants received the same amounts of water and light. The plants were divided into three groups, and each was exposed to a different concentration of CO₂ in the air: 350, 600, or 1,000 ppm (parts per million).

Data from the Experiment The table shows the dry mass (in grams) of corn and velvetleaf plants grown at the three concentrations of CO₂. The dry mass values are averages of the leaves, stems, and roots of eight plants.

	350 ppm CO ₂	600 ppm CO ₂	1,000 ppm CO ₂
Average dry mass of one corn plant (g)	91	89	80
Average dry mass of one velvetleaf plant (g)	35	48	54

Data from D. T. Patterson and E. P. Flint, Potential effects of global atmospheric CO₂ enrichment on the growth and competitiveness of C₃ and C₄ weed and crop plants, *Weed Science* 28(1):71–75 (1980).

INTERPRET THE DATA

- To explore the relationship between the two variables, it is useful to graph the data in a scatter plot, and then draw a regression line.
(a) First, place labels for the dependent and independent variables on the appropriate axes. Explain your choices. (b) Now plot the data points for corn and velvetleaf using different symbols for each set of data, and add a key for the two symbols. (For additional information about graphs, see the Scientific Skills Review in Appendix F.)
- Draw a “best-fit” line for each set of points. A best-fit line does not necessarily pass through all or even most points. Instead, it is a straight line that passes as close as possible to all data points from that set. Draw a best-fit line for each set of data. Because placement of the line is a matter of judgment, two individuals may draw two slightly different lines for a given set of points.

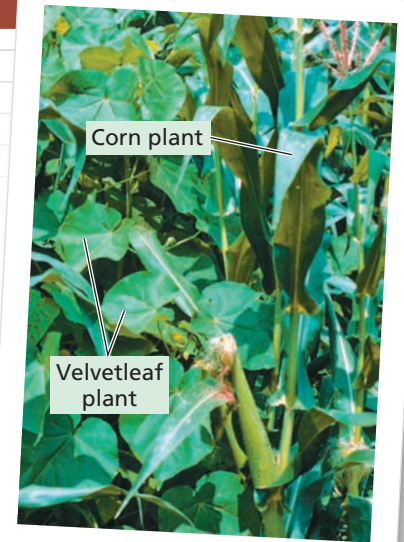
➤ Corn plant surrounded by invasive velvetleaf plants

The line that actually fits best, a regression line, can be identified by squaring the distances of all points to any candidate line, then selecting the line that minimizes the sum of the squares. (See the graph in the Scientific Skills Exercise in Chapter 3 for an example of a linear regression line.)

Excel or other software programs, including those on a graphing calculator, can plot a regression line once data points are entered. Using either Excel or a graphing calculator, enter the data points for each data set and have the program draw the two regression lines. Compare them to the lines you drew.

- Describe the trends shown by the regression lines in your scatter plot. (a) Compare the relationship between increasing concentration of CO₂ and the dry mass of corn to that for velvetleaf. (b) Considering that velvetleaf is a weed invasive to cornfields, predict how increased CO₂ concentration may affect interactions between the two species.
- Based on the data in the scatter plot, estimate the percentage change in dry mass of corn and velvetleaf plants if atmospheric CO₂ concentration increased from 390 ppm (current levels) to 800 ppm. (a) What is the estimated dry mass of corn and velvetleaf plants at 390 ppm? 800 ppm? (b) To calculate the percentage change in mass for each plant, subtract the mass at 390 ppm from the mass at 800 ppm (change in mass), divide by the mass at 390 ppm (initial mass), and multiply by 100. What is the estimated percentage change in dry mass for corn? For velvetleaf? (c) Do these results support the conclusion from other experiments that C₃ plants grow better than C₄ plants under increased CO₂ concentration? Why or why not?

Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.



C₄ photosynthesis is considered more efficient than C₃ photosynthesis because it uses less water and resources. On our planet today, the world population and demand for food are rapidly increasing. At the same time, the amount of land suitable for growing crops is decreasing due to the effects of global climate change, which include an increase in sea level as well as a hotter, drier climate in many regions. To address issues of food supply, scientists in the Philippines have been working on genetically modifying rice—an important food staple that is a C₃ crop—so that it can instead carry out C₄ photosynthesis. Results so far seem promising, and these researchers estimate

that the yield of C₄ rice might be 30–50% higher than C₃ rice with the same input of water and resources.

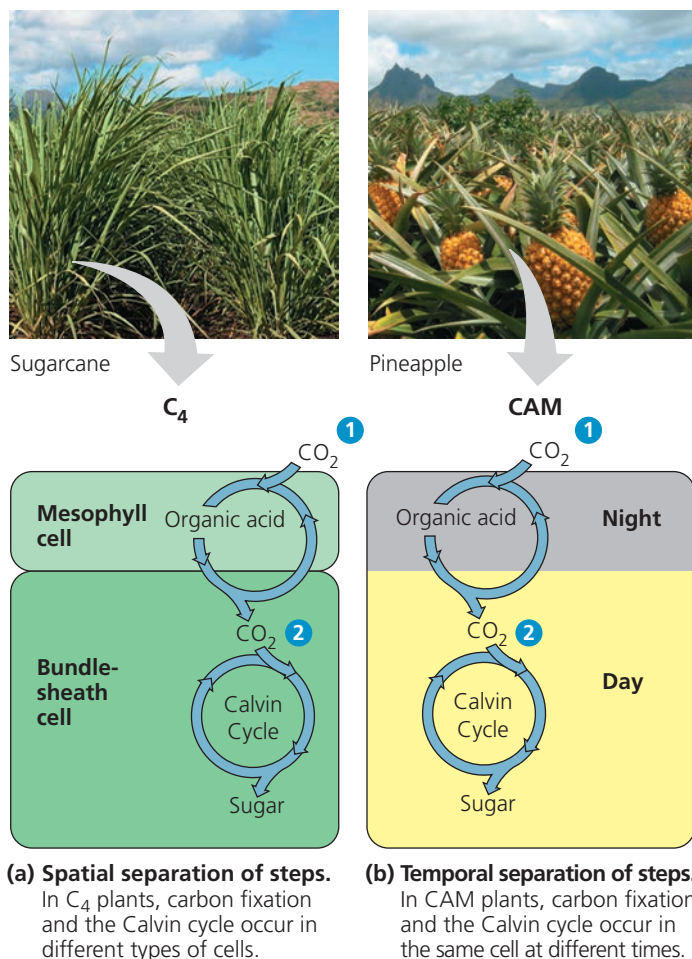
CAM Plants

A second photosynthetic adaptation to arid conditions has evolved in many succulent (water-storing) plants, numerous cacti, pineapples, and representatives of several other plant families. These plants open their stomata during the night and close them during the day, just the reverse of how other plants behave. Closing stomata during the day helps desert plants conserve water, but it also prevents CO₂ from

entering the leaves. During the night, when their stomata are open, these plants take up CO_2 and incorporate it into a variety of organic acids. This mode of carbon fixation is called **crassulacean acid metabolism**, or **CAM**, after the plant family Crassulaceae, the succulents in which the process was first discovered. The mesophyll cells of **CAM plants** store the organic acids they make during the night in their vacuoles until morning, when the stomata close. During the day, when the light reactions can supply ATP and NADPH for the Calvin cycle, CO_2 is released from the organic acids made the night before to become incorporated into sugar in the chloroplasts.

Notice in **Figure 11.21** that the CAM pathway is similar to the C_4 pathway in that CO_2 is first incorporated into organic intermediates before it enters the Calvin cycle. The difference is that in C_4 plants, the initial steps of carbon fixation are separated structurally from the Calvin cycle, whereas in CAM plants, the two steps occur within the same cell but at separate times. (Keep in mind that CAM, C_4 , and C_3 plants all eventually use the Calvin cycle to make sugar from carbon dioxide.)

▼ **Figure 11.21 C_4 and CAM photosynthesis compared.** The C_4 and CAM pathways are two evolutionary solutions to the problem of maintaining photosynthesis with stomata partially or completely closed on hot, dry days. Both adaptations are characterized by **1** preliminary incorporation of CO_2 into organic acids, followed by **2** transfer of CO_2 to the Calvin cycle.



Animation: Photosynthesis in Dry Climates

CONCEPT CHECK 11.4

1. Describe how photorespiration lowers photosynthetic output for plants.
2. The presence of only PS I, not PS II, in the bundle-sheath cells of C_4 plants has an effect on O_2 concentration. What is that effect, and how might that benefit the plant?
3. **MAKE CONNECTIONS** ▶ Refer to the discussion of ocean acidification in Concept 3.3. Ocean acidification and changes in the distribution of C_3 and C_4 plants may seem to be two very different problems, but what do they have in common? Explain.
4. **WHAT IF?** ▶ A CAM plant is placed in a controlled environment, where the plant is grown in the constant presence of light. How would this continuous exposure to light affect the plant?

For suggested answers, see Appendix A.

CONCEPT 11.5

Life depends on photosynthesis

The Importance of Photosynthesis: A Review

In this chapter, we have followed photosynthesis from photons to food. The light reactions capture solar energy and use it to make ATP and transfer electrons from water to NADP^+ , forming NADPH. The Calvin cycle uses the ATP and NADPH to produce sugar from carbon dioxide. The energy that enters the chloroplasts as sunlight becomes stored as chemical energy in organic compounds. The entire process is reviewed visually in **Figure 11.22**, where photosynthesis is also put in its natural context.

As for the fates of photosynthetic products, enzymes in the chloroplast and cytosol convert the G3P made in the Calvin cycle to many other organic compounds. In fact, the sugar made in the chloroplasts supplies the entire plant with chemical energy and carbon skeletons for the synthesis of all the major organic molecules of plant cells. About 50% of the organic material made by photosynthesis is consumed as fuel for cellular respiration in plant cell mitochondria.

Technically, green cells are the only autotrophic parts of the plant. The rest of the plant depends on organic molecules exported from leaves via veins (see Figure 11.22, top). In most plants, carbohydrate is transported out of the leaves to the rest of the plant in the form of sucrose, a disaccharide. After arriving at nonphotosynthetic cells, the sucrose provides raw material for cellular respiration and a multitude of anabolic pathways that synthesize proteins, lipids, and other products. A considerable amount of sugar in the form of glucose is linked together to make the polysaccharide cellulose (see Figure 5.6c), especially in plant cells that are still growing and maturing. Cellulose, the main ingredient of cell walls, is the most abundant organic molecule in the plant—and probably on the surface of the planet.

Most plants and other photosynthesizers make more organic material each day than they need to use as respiratory

fuel and precursors for biosynthesis. They stockpile the extra sugar by synthesizing starch, storing some in the chloroplasts themselves and some in storage cells of roots, tubers, seeds, and fruits. In accounting for the consumption of the food molecules produced by photosynthesis, let's not forget that most plants lose leaves, roots, stems, fruits, and sometimes their entire bodies to heterotrophs, including humans.

On a global scale, photosynthesis is the process responsible for the presence of oxygen in our atmosphere. Furthermore, although each chloroplast is minuscule, their collective productivity in terms of food production is prodigious: Photosynthesis makes an estimated 150 billion metric tons of carbohydrate per year (a metric ton is 1,000 kg, about 1.1 tons). That's organic matter equivalent in mass to a stack of about 60 trillion biology textbooks—and the stack

would reach 17 times the distance from Earth to the sun! No chemical process is more important than photosynthesis to the welfare of life on Earth.

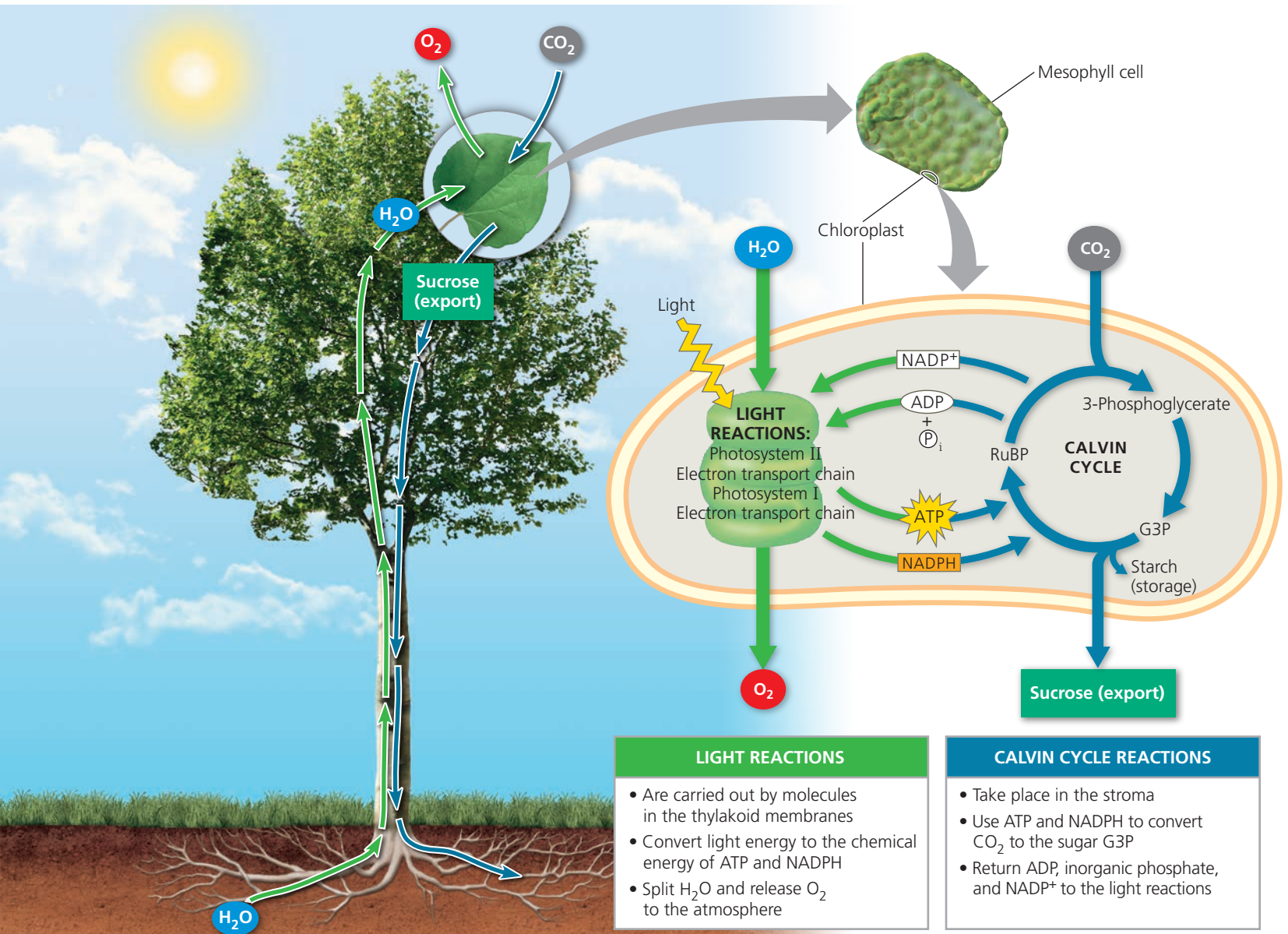
In Chapters 5 through 8 and 10, 11, you have learned about many activities of cells. **Figure 11.23** integrates these cellular processes into the context of a working plant cell. As you study the figure, reflect on how each process fits into the big picture: As the most basic unit of living organisms, a cell performs all functions characteristic of life.

CONCEPT CHECK 11.5

- 1. MAKE CONNECTIONS** > Can plants use the sugar they produce during photosynthesis to directly power the work of the cell? Explain. (See Figures 6.10, 6.11, and 10.6.)

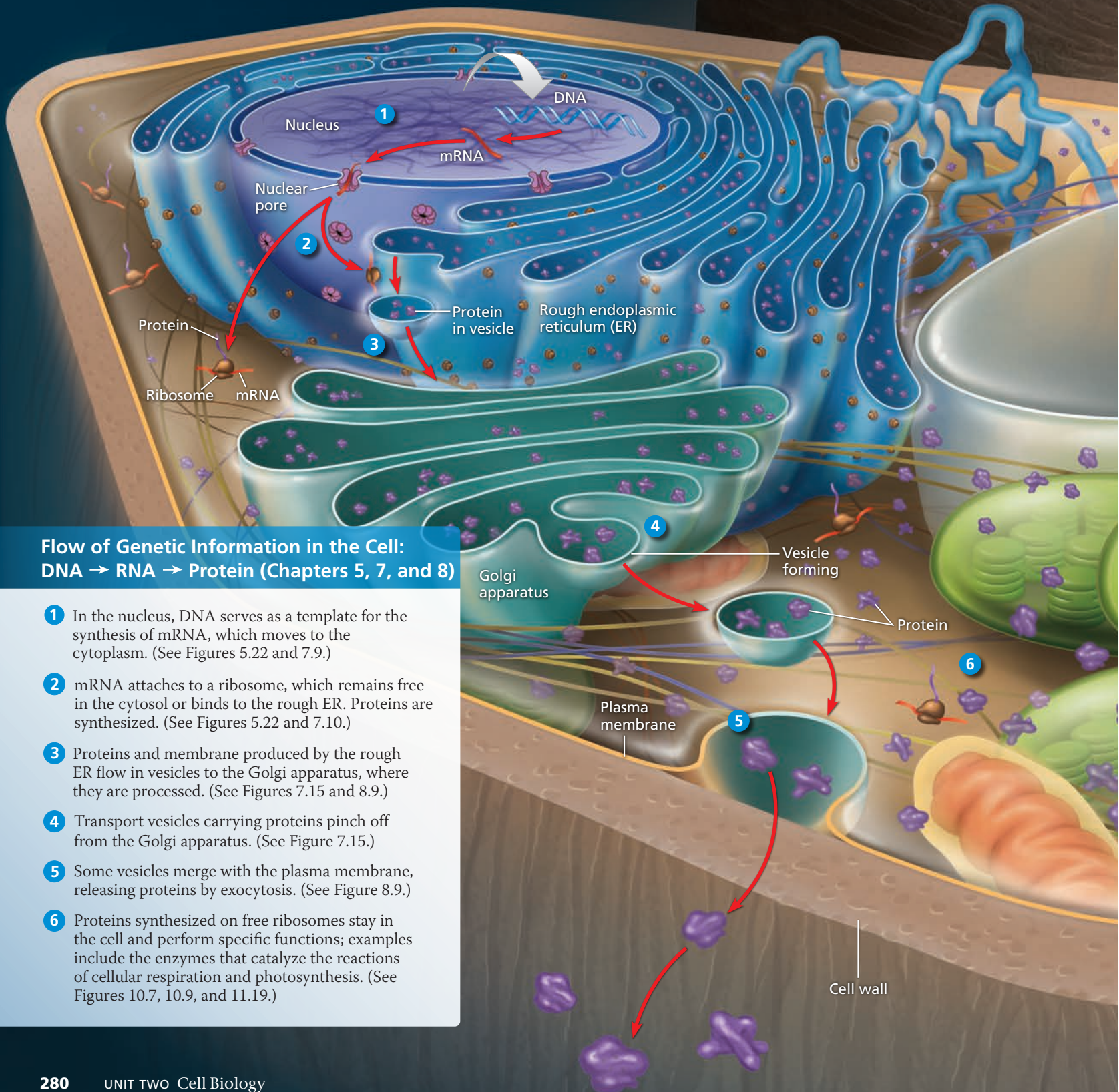
For suggested answers, see Appendix A.

▼ **Figure 11.22 A review of photosynthesis.** This diagram shows the main reactants and products of photosynthesis as they move through the tissues of a tree (left) and a chloroplast (right).



The Working Cell

This figure illustrates how a generalized plant cell functions, integrating the cellular activities you learned about in Chapters 5–11.



Flow of Genetic Information in the Cell: DNA → RNA → Protein (Chapters 5, 7, and 8)

- 1** In the nucleus, DNA serves as a template for the synthesis of mRNA, which moves to the cytoplasm. (See Figures 5.22 and 7.9.)
- 2** mRNA attaches to a ribosome, which remains free in the cytosol or binds to the rough ER. Proteins are synthesized. (See Figures 5.22 and 7.10.)
- 3** Proteins and membrane produced by the rough ER flow in vesicles to the Golgi apparatus, where they are processed. (See Figures 7.15 and 8.9.)
- 4** Transport vesicles carrying proteins pinch off from the Golgi apparatus. (See Figure 7.15.)
- 5** Some vesicles merge with the plasma membrane, releasing proteins by exocytosis. (See Figure 8.9.)
- 6** Proteins synthesized on free ribosomes stay in the cell and perform specific functions; examples include the enzymes that catalyze the reactions of cellular respiration and photosynthesis. (See Figures 10.7, 10.9, and 11.19.)

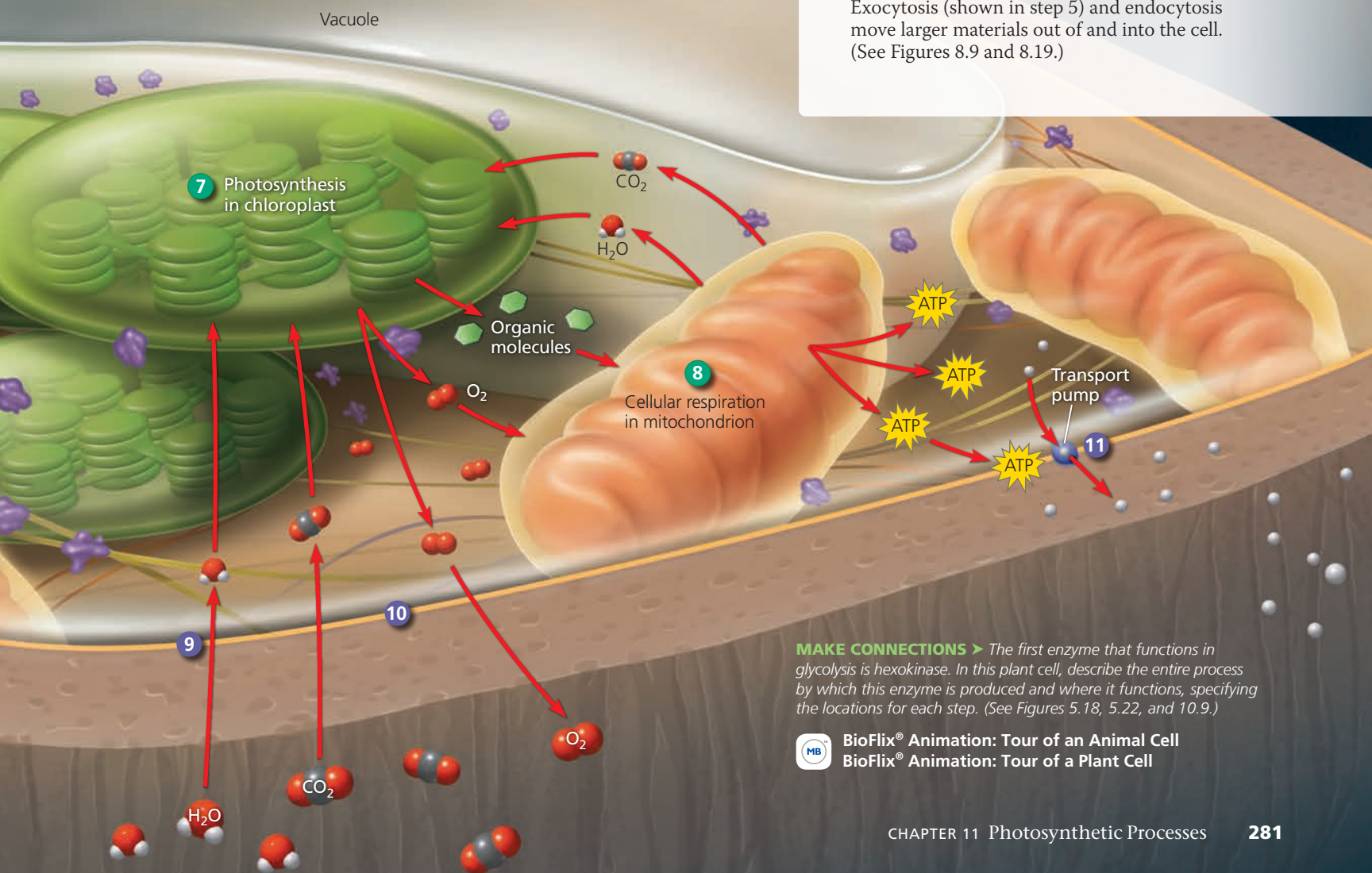
Energy Transformations in the Cell: Photosynthesis and Cellular Respiration (Chapters 6, 10 and 11)

- 7** In chloroplasts, the process of photosynthesis uses the energy of light to convert CO_2 and H_2O to organic molecules, with O_2 as a by-product. (See Figure 11.22.)
- 8** In mitochondria, organic molecules are broken down by cellular respiration, capturing energy in molecules of ATP, which are used to power the work of the cell, such as protein synthesis and active transport. CO_2 and H_2O are by-products. (See Figures 6.9–6.11, 10.2, and 10.16.)

Movement Across Cell Membranes (Chapter 8)

- 9** Water diffuses into and out of the cell directly through the plasma membrane and by facilitated diffusion through aquaporins. (See Figure 8.1.)
- 10** By passive transport, the CO_2 used in photosynthesis diffuses into the cell and the O_2 formed as a by-product of photosynthesis diffuses out of the cell. Both solutes move down their concentration gradients. (See Figures 8.10 and 11.22.)
- 11** In active transport, energy (usually supplied by ATP) is used to transport a solute against its concentration gradient. (See Figure 8.16.)

Exocytosis (shown in step 5) and endocytosis move larger materials out of and into the cell. (See Figures 8.9 and 8.19.)



MAKE CONNECTIONS ▶ The first enzyme that functions in glycolysis is hexokinase. In this plant cell, describe the entire process by which this enzyme is produced and where it functions, specifying the locations for each step. (See Figures 5.18, 5.22, and 10.9.)



BioFlix® Animation: Tour of an Animal Cell
BioFlix® Animation: Tour of a Plant Cell

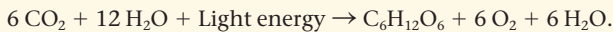


SUMMARY OF KEY CONCEPTS

CONCEPT 11.1

Photosynthesis converts light energy to the chemical energy of food (pp. 261–264)

- In eukaryotes that are **autotrophs**, photosynthesis occurs in **chloroplasts**, organelles containing **thylakoids**. Stacks of thylakoids form grana. **Photosynthesis** is summarized as



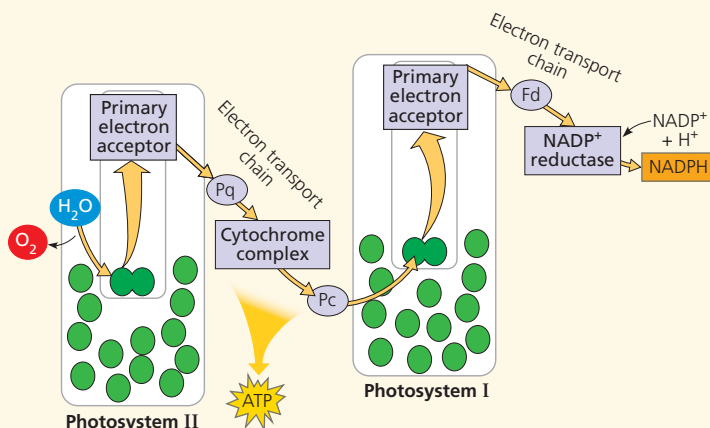
Chloroplasts split water into hydrogen and oxygen, incorporating the electrons of hydrogen into sugar molecules. Photosynthesis is a redox process: H_2O is oxidized, and CO_2 is reduced. The **light reactions** in the thylakoid membranes split water, releasing O_2 , producing ATP, and forming **NADPH**. The **Calvin cycle** in the **stroma** forms sugar from CO_2 , using ATP for energy and NADPH for reducing power.

- ?** Compare the roles of CO_2 and H_2O in cellular respiration and photosynthesis.

CONCEPT 11.2

The light reactions convert solar energy to the chemical energy of ATP and NADPH (pp. 264–273)

- Light is a form of electromagnetic energy. The colors we see as **visible light** include those **wavelengths** that drive photosynthesis. A pigment absorbs light of specific wavelengths; **chlorophyll *a*** is the main photosynthetic pigment in plants. Other accessory pigments absorb different wavelengths of light and pass the energy on to chlorophyll *a*.
- A pigment goes from a ground state to an excited state when a **photon** of light boosts one of the pigment's electrons to a higher-energy orbital. This excited state is unstable. Electrons from isolated pigments tend to fall back to the ground state, giving off heat and/or light.
- A **photosystem** is composed of a **reaction-center complex** surrounded by **light-harvesting complexes** that funnel the energy of photons to the reaction-center complex. When a special pair of reaction-center chlorophyll *a* molecules absorbs energy, one of its electrons is boosted to a higher energy level and transferred to the **primary electron acceptor**. **Photosystem II** contains P680 chlorophyll *a* molecules in the reaction-center complex; **photosystem I** contains P700 molecules.
- Linear electron flow** during the light reactions uses both photosystems and produces NADPH, ATP, and oxygen:



VOCAB
SELF-QUIZ
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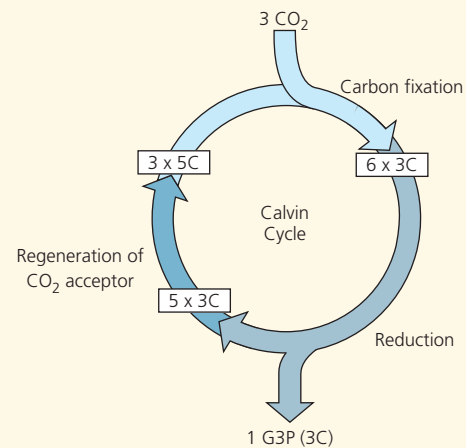
- Cyclic electron flow** employs only one photosystem, producing ATP but no NADPH or O_2 .
- During chemiosmosis in both mitochondria and chloroplasts, electron transport chains generate an H^+ gradient across a membrane. ATP synthase uses this proton-motive force to make ATP.

- ?** The absorption spectrum of chlorophyll *a* differs from the action spectrum of photosynthesis. Explain this observation.

CONCEPT 11.3

The Calvin cycle uses the chemical energy of ATP and NADPH to reduce CO_2 to sugar (pp. 273–274)

- The Calvin cycle occurs in the stroma, using electrons from NADPH and energy from ATP. One molecule of **G3P** exits the cycle per three CO_2 molecules fixed and is converted to glucose and other organic molecules.



- DRAW IT >** On the diagram above, draw where ATP and NADPH are used and where rubisco functions. Describe these steps.

CONCEPT 11.4

Alternative mechanisms of carbon fixation have evolved in hot, arid climates (pp. 275–278)

- On dry, hot days, **C_3 plants** close their stomata, conserving water but keeping CO_2 out and O_2 in. Under these conditions, **photorespiration** can occur: **Rubisco** binds O_2 instead of CO_2 , consuming ATP and releasing CO_2 without producing ATP or carbohydrate. Photorespiration may be an evolutionary relic, and it may play a photoprotective role.
- C_4 plants** minimize the cost of photorespiration by incorporating CO_2 into four-carbon compounds in mesophyll cells. These compounds are exported to **bundle-sheath cells**, where they release carbon dioxide for use in the Calvin cycle.
- CAM plants** open their stomata at night, incorporating CO_2 into organic acids, which are stored in mesophyll cells. During the day, the stomata close, and the CO_2 is released from the organic acids for use in the Calvin cycle.
- Organic compounds produced by photosynthesis provide the energy and building material for Earth's ecosystems.

- ?** Why are C_4 and CAM photosynthesis more energetically expensive than C_3 photosynthesis? What climate conditions would favor C_4 and CAM plants?

CONCEPT 11.5

Life depends on photosynthesis (pp. 278–279)

- Organic compounds produced by photosynthesis provide the energy and building material for Earth's ecosystems.

 Explain how all life depends on photosynthesis.

TEST YOUR UNDERSTANDING



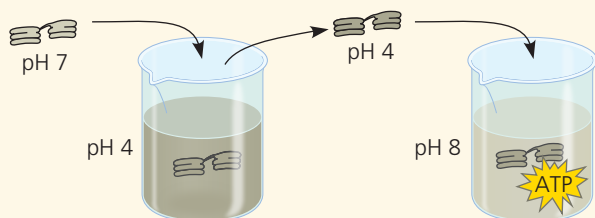
Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. SCIENCE, TECHNOLOGY, AND SOCIETY

Scientific evidence indicates that the CO_2 added to the air by the burning of wood and fossil fuels is contributing to global warming, a rise in global temperature. Tropical rain forests are estimated to be responsible for approximately 20% of global photosynthesis, yet the consumption of large amounts of CO_2 by living trees is thought to make little or no *net* contribution to reduction of global warming. Explain why this might be the case. (*Hint*: What processes in both living and dead trees produce CO_2 ?)

9. **EVOLUTION CONNECTION** Photorespiration can decrease soybeans' photosynthetic output by about 50%. Would you expect this figure to be higher or lower in wild relatives of soybeans? Why?

10. **SCIENTIFIC INQUIRY • MAKE CONNECTIONS** The following diagram represents an experiment with isolated thylakoids. The thylakoids were first made acidic by soaking them in a solution at pH 4. After the thylakoid space reached pH 4, the thylakoids were transferred to a basic solution at pH 8. The thylakoids then made ATP in the dark. (See Concept 3.3 to review pH.)



PRACTICE
TEST
goo.gl/iAsVgI

Draw an enlargement of part of the thylakoid membrane in the beaker with the solution at pH 8. Draw ATP synthase. Label the areas of high H^+ concentration and low H^+ concentration. Show the direction protons flow through the enzyme, and show the reaction where ATP is synthesized. Would ATP end up in the thylakoid or outside of it? Explain why the thylakoids in the experiment were able to make ATP in the dark.

11. **WRITE ABOUT A THEME: ENERGY AND MATTER** Life is solar powered. Almost all the producers of the biosphere depend on energy from the sun to produce the organic molecules that supply the energy and carbon skeletons needed for life. In a short essay (100–150 words), describe how the process of photosynthesis in the chloroplasts of plants transforms the energy of sunlight into the chemical energy of sugar molecules.

12. SYNTHESIZE YOUR KNOWLEDGE

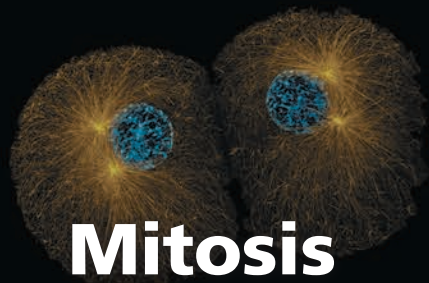


“Watermelon snow” in Antarctica is caused by a species of photosynthetic green algae that thrives in sub-zero temperatures (*Chlamydomonas nivalis*). These algae are also found in high-altitude year-round snowfields. In both locations, UV light levels tend to be high. Based on what you learned in this chapter, propose an explanation for why this photosynthetic alga appears reddish-pink.

For selected answers, see Appendix A.

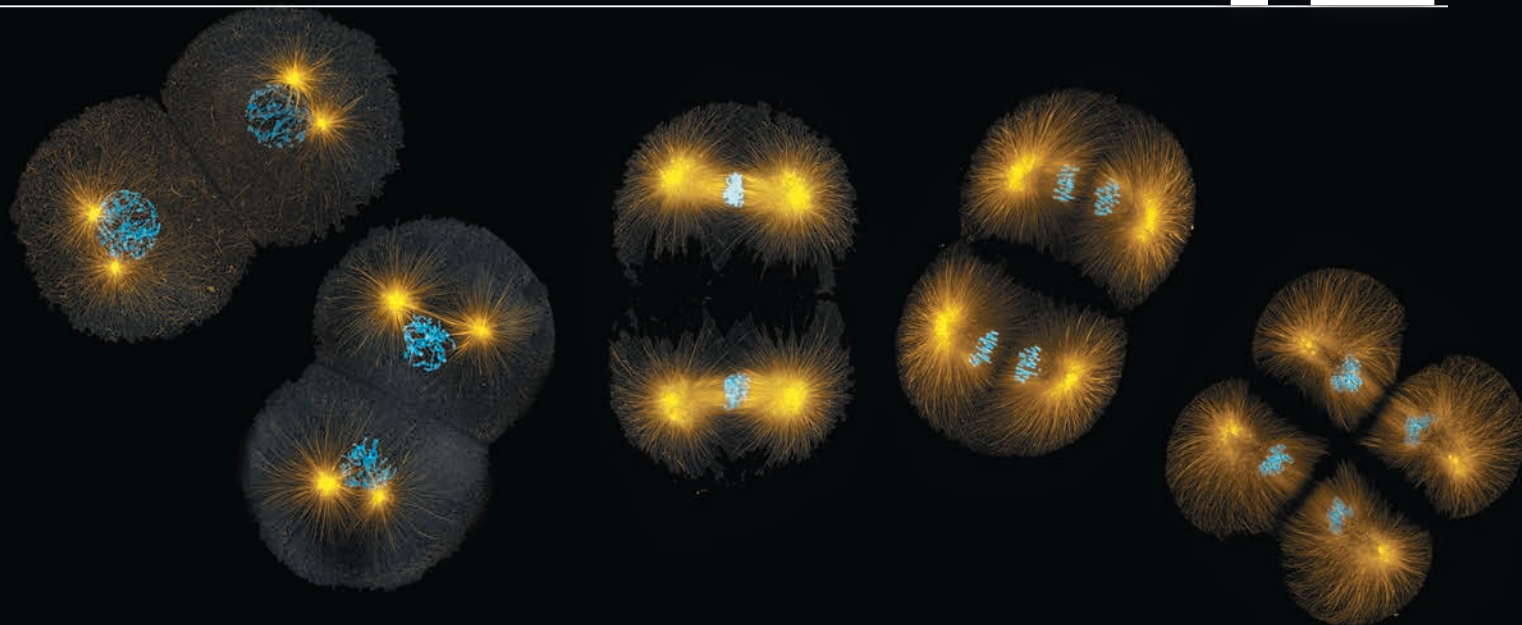


For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!



Mitosis

12

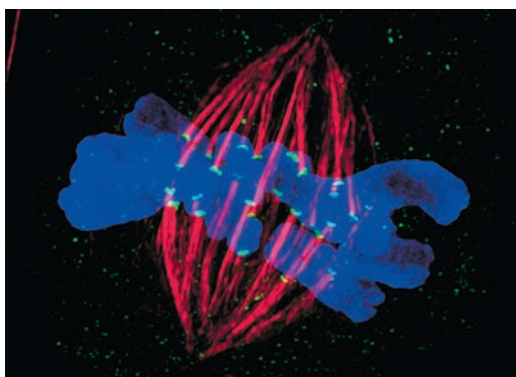


▲ **Figure 12.1** How do dividing cells distribute chromosomes to daughter cells?

KEY CONCEPTS

- 12.1** Most cell division results in genetically identical daughter cells
- 12.2** The mitotic phase alternates with interphase in the cell cycle
- 12.3** The eukaryotic cell cycle is regulated by a molecular control system

▼ Chromosomes (blue) are attached by specific proteins (green) to cell machinery (red) and are moved during division of a rat kangaroo cell.



The Key Roles of Cell Division

The ability of organisms to produce more of their own kind is the one characteristic that best distinguishes living things from nonliving matter. This unique capacity to procreate, like all biological functions, has a cellular basis. In 1855, Rudolf Virchow, a German physician, put it this way: “Where a cell exists, there must have been a pre-existing cell, just as the animal arises only from an animal and the plant only from a plant.” He summarized this concept with the Latin saying “*Omnis cellula e cellula*,” meaning “Every cell from a cell.” The continuity of life is based on the reproduction of cells, or **cell division**. The series of confocal fluorescence micrographs in **Figure 12.1**, starting at the upper left and reading from left to right, follows the events of cell division as the cells of a two-celled marine worm embryo become four.

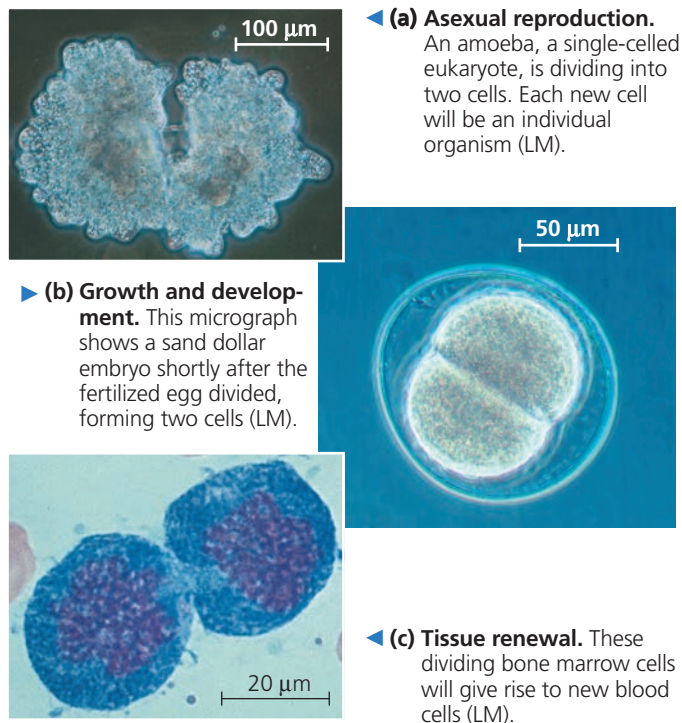
Cell division plays several important roles in life. When a prokaryotic cell divides, it is actually reproducing, since the process gives rise to a new organism (another cell). The same is true of any unicellular eukaryote, such as the amoeba shown in **Figure 12.2a**. As for multicellular eukaryotes, cell division enables each of these organisms to develop from a single cell—the fertilized egg. A two-celled embryo, the first stage in this process, is shown in **Figure 12.2b**. And cell division continues to function in renewal and repair in fully grown multicellular eukaryotes, replacing cells that die from accidents or normal wear and tear. For example, dividing cells in your bone marrow continuously make new blood cells (**Figure 12.2c**).

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

▼ **Figure 12.2 The functions of cell division.**



Cell Division in a Sea Urchin Embryo

The cell division process is an integral part of the **cell cycle**, the life of a cell from the time it is first formed during division of a parent cell until its own division into two daughter cells. (Biologists use the words *daughter* or *sister* in relation to cells, but this is not meant to imply gender.) Passing identical genetic material to cellular offspring is a crucial function of cell division. In this chapter, you will learn how this process occurs in the context of the cell cycle. After studying the cellular mechanics of cell division in eukaryotes and bacteria, you will learn about the molecular control system that regulates progress through the eukaryotic cell cycle and what happens when the control system malfunctions. Because a breakdown in cell cycle control plays a major role in the development of cancer, this aspect of cell biology is an active area of research.

CONCEPT 12.1

Most cell division results in genetically identical daughter cells

The reproduction of a cell, with all of its complexity, cannot occur by a mere pinching in half; a cell is not like a soap bubble that simply enlarges and splits in two. In both prokaryotes and eukaryotes, most cell division involves the distribution of identical genetic material—DNA—to two daughter cells. (The exception is meiosis, the special type of eukaryotic cell division that can produce sperm and eggs.) What is most remarkable about cell division is the accuracy with which the DNA

is passed from one generation of cells to the next. A dividing cell replicates its DNA, distributes the two copies to opposite ends of the cell, and then splits into daughter cells.

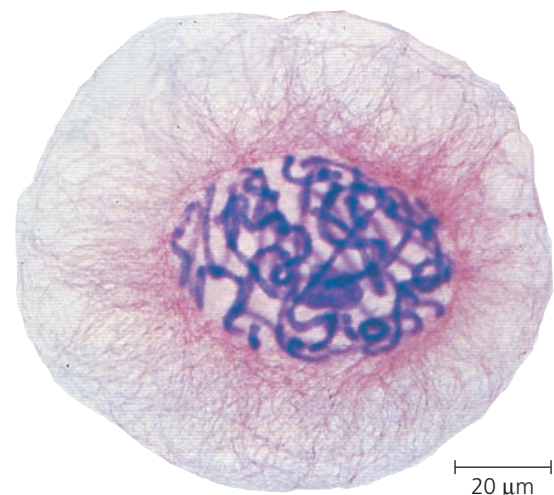
Cellular Organization of the Genetic Material

A cell's DNA, its genetic information, is called its **genome**. Although a prokaryotic genome is often a single DNA molecule, eukaryotic genomes usually consist of a number of DNA molecules. The overall length of DNA in a eukaryotic cell is enormous. A typical human cell, for example, has about 2 m of DNA—a length about 250,000 times greater than the cell's diameter. Before the cell can divide to form genetically identical daughter cells, all of this DNA must be copied, or replicated, and then the two copies must be separated so that each daughter cell ends up with a complete genome.

The replication and distribution of so much DNA are manageable because the DNA molecules are packaged into structures called **chromosomes** (from the Greek *chroma*, color, and *soma*, body), so named because they take up certain dyes used in microscopy (**Figure 12.3**). Each eukaryotic chromosome consists of one very long, linear DNA molecule associated with many proteins (see Figure 7.9). The DNA molecule carries several hundred to a few thousand genes, the units of information that specify an organism's inherited traits. The associated proteins maintain the structure of the chromosome and help control the activity of the genes. Together, the entire complex of DNA and proteins that is the building material of chromosomes is referred to as **chromatin**. As you will soon see, the chromatin of a chromosome varies in its degree of condensation during the process of cell division.

Every eukaryotic species has a characteristic number of chromosomes in each cell's nucleus. For example, the nuclei of human **somatic cells** (all body cells except the reproductive cells) each contain 46 chromosomes, made up of two sets

▼ **Figure 12.3 Eukaryotic chromosomes.** Chromosomes (stained purple) are visible within the nucleus of this cell from an African blood lily. The thinner red threads in the surrounding cytoplasm are the cytoskeleton. The cell is preparing to divide (LM).



of 23, one set inherited from each parent. Reproductive cells, or **gametes**—such as sperm and eggs—have half as many chromosomes as somatic cells; in our example, human gametes have one set of 23 chromosomes. The number of chromosomes in somatic cells varies widely among species: 18 in cabbage plants, 48 in chimpanzees, 56 in elephants, 90 in hedgehogs, and 148 in one species of alga. We'll now consider how these chromosomes behave during cell division.

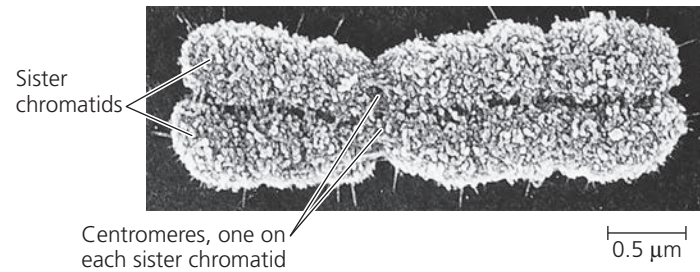
Distribution of Chromosomes During Eukaryotic Cell Division

When a cell is not dividing, and even as it replicates its DNA in preparation for cell division, each chromosome is in the form of a long, thin chromatin fiber. After DNA replication, however, the chromosomes condense as a part of cell division: Each chromatin fiber becomes densely coiled and folded, making the chromosomes much shorter and so thick that we can see them with a light microscope.

Each duplicated chromosome consists of two **sister chromatids**, which are joined copies of the original chromosome (**Figure 12.4**). The two chromatids, each containing an identical DNA molecule, are typically attached all along their lengths by protein complexes called *cohesins*; this attachment is known as *sister chromatid cohesion*. Each sister chromatid has a **centromere**, a region made up of repetitive sequences in the chromosomal DNA where the chromatid is attached most closely to its sister chromatid. This attachment is mediated by proteins that recognize and bind to the centromeric DNA; other bound proteins condense the DNA, giving the duplicated chromosome a narrow “waist.” The portion of a chromatid to either side of the centromere is referred to as an *arm* of the chromatid. (An unduplicated chromosome has a single centromere, distinguished by the proteins that bind there, and two arms.)

Later in the cell division process, the two sister chromatids of each duplicated chromosome separate and move into two new nuclei, one forming at each end of the cell. Once the sister chromatids separate, they are no longer called sister chromatids but are considered individual chromosomes; this is the step that essentially doubles the number of chromosomes during cell division. Thus, each new nucleus receives a collection of

▼ **Figure 12.4** A highly condensed, duplicated human chromosome (SEM).

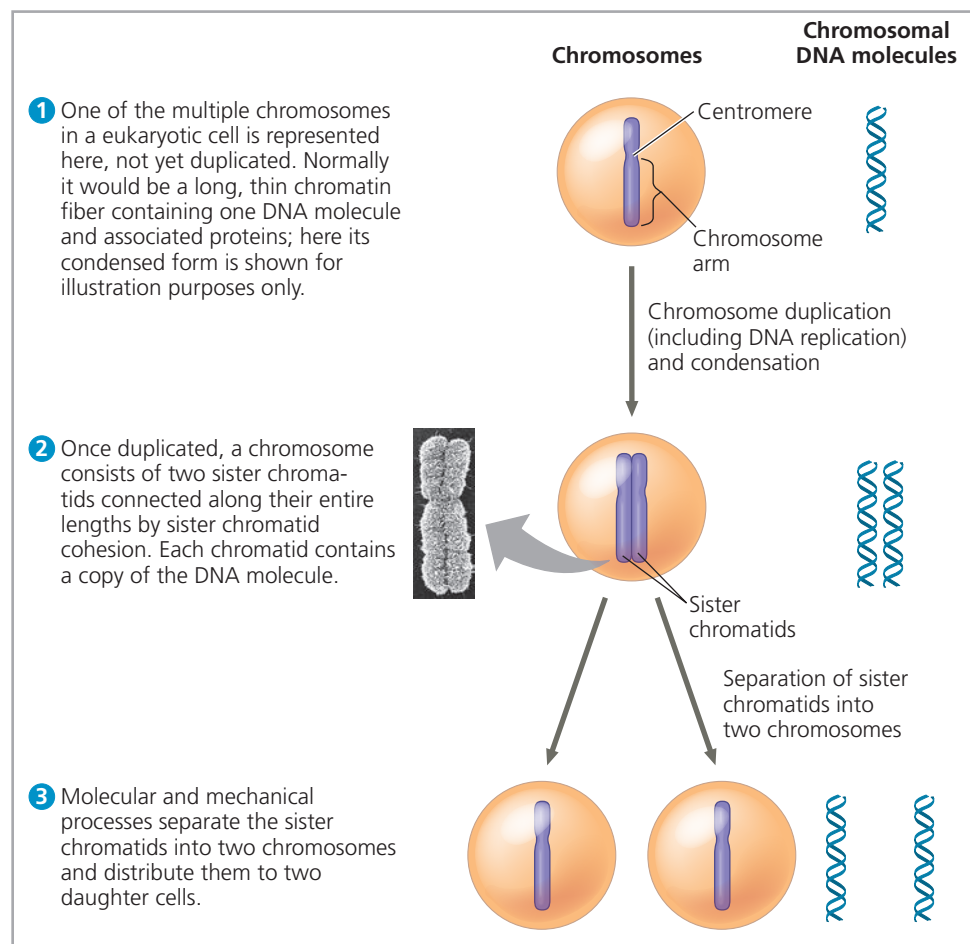


DRAW IT ► Circle one sister chromatid of the chromosome in this micrograph.

chromosomes identical to that of the parent cell (**Figure 12.5**). **Mitosis**, the division of the genetic material in the nucleus, is usually followed immediately by **cytokinesis**, the division of the cytoplasm. One cell has become two, each the genetic equivalent of the parent cell.

From a fertilized egg, mitosis and cytokinesis produced the 200 trillion somatic cells that now make up your body, and the same processes continue to generate new cells to

▼ **Figure 12.5** Chromosome duplication and distribution during cell division.



? How many chromatid arms does the chromosome in 2 have? Identify the point in the figure where one chromosome becomes two.

BioFlix® Animation: Chromosome Duplication

replace dead and damaged ones. In contrast, you produce gametes—eggs or sperm—by a variation of cell division called *meiosis*, which yields daughter cells with only one set of chromosomes, half as many chromosomes as the parent cell. Meiosis in humans occurs only in special cells in the ovaries or testes (the gonads). Generating gametes, meiosis reduces the chromosome number from 46 (two sets) to 23 (one set). Fertilization fuses two gametes together and returns the chromosome number to 46 (two sets). Mitosis then conserves that number in every somatic cell nucleus of the new human individual. In Chapter 13, we will examine the role of meiosis in reproduction and inheritance in more detail. In the remainder of this chapter, we focus on mitosis and the rest of the cell cycle in eukaryotes.

CONCEPT CHECK 12.1

1. How many chromosomes are drawn in each part of Figure 12.5? (Ignore the micrograph in step 2.)
2. **WHAT IF? >** A mother kangaroo has 16 chromosomes in its somatic cells. How many chromosomes will be in a fertilized egg of the kangaroo? How many chromosomes will it pass on to its offspring? How many chromosomes will the offspring inherit from the father kangaroo?

For suggested answers, see Appendix A.

CONCEPT 12.2

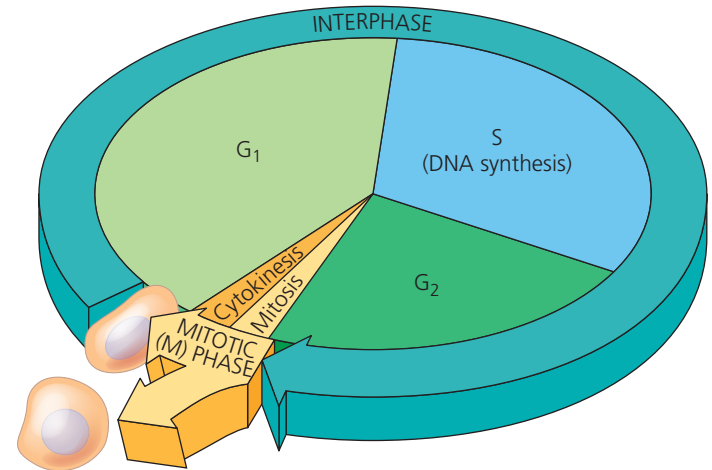
The mitotic phase alternates with interphase in the cell cycle

In 1882, a German anatomist named Walther Flemming developed dyes that allowed him to observe, for the first time, the behavior of chromosomes during mitosis and cytokinesis. (In fact, Flemming coined the terms *mitosis* and *chromatin*.) During the period between one cell division and the next, it appeared to Flemming that the cell was simply growing larger. But we now know that many critical events occur during this stage in the life of a cell.

Phases of the Cell Cycle

Mitosis is just one part of the cell cycle (**Figure 12.6**). In fact, the **mitotic (M) phase**, which includes both mitosis and cytokinesis, is usually the shortest part of the cell cycle. The mitotic phase alternates with a much longer stage called **interphase**, which often accounts for about 90% of the cycle. Interphase can be divided into three phases: the **G₁ phase** (“first gap”), the **S phase** (“synthesis”), and the **G₂ phase** (“second gap”). The G phases were misnamed as “gaps” when they were first observed because the cells appeared inactive, but we now know that intense metabolic activity and growth occur throughout interphase. During all three phases of interphase, in fact, a cell grows by producing proteins and cytoplasmic organelles such as mitochondria and endoplasmic reticulum. Duplication of

✓ Figure 12.6 The cell cycle. In a dividing cell, the mitotic (M) phase alternates with interphase, a growth period. The first part of interphase (G₁) is followed by the S phase, when the chromosomes duplicate; G₂ is the last part of interphase. In the M phase, mitosis distributes the daughter chromosomes to daughter nuclei, and cytokinesis divides the cytoplasm, producing two daughter cells.



Animation: The Cell Cycle

the chromosomes, crucial for eventual division of the cell, occurs entirely during the S phase. (We will discuss synthesis, or replication, of DNA in Concept 16.2.) Thus, a cell grows (G₁), continues to grow as it copies its chromosomes (S), grows more as it completes preparations for cell division (G₂), and divides (M). The daughter cells may then repeat the cycle.

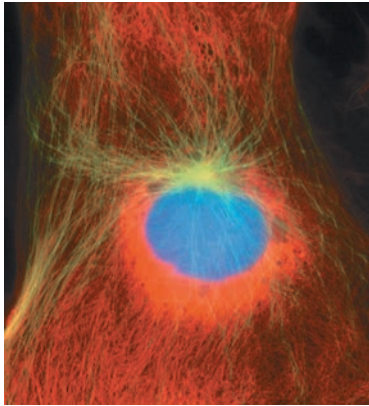
A particular human cell might undergo one division in 24 hours. Of this time, the M phase would occupy less than 1 hour, while the S phase might occupy 10–12 hours, or about half the cycle. The rest of the time would be apportioned between the G₁ and G₂ phases. The G₂ phase usually takes 4–6 hours; in our example, G₁ would occupy about 5–6 hours. G₁ is the most variable in length in different types of cells. Some cells in a multicellular organism divide very infrequently or not at all. These cells spend their time in G₁ (or a related phase called G₀, to be discussed later in the chapter) doing their job in the organism—a cell of the pancreas secretes digestive enzymes, for example.

Mitosis is conventionally broken down into five stages: **prophase**, **prometaphase**, **metaphase**, **anaphase**, and **telophase**. Overlapping with the latter stages of mitosis, cytokinesis completes the mitotic phase. **Figure 12.7** describes these stages in an animal cell. Study this figure thoroughly before progressing to the next two sections, which examine mitosis and cytokinesis more closely.

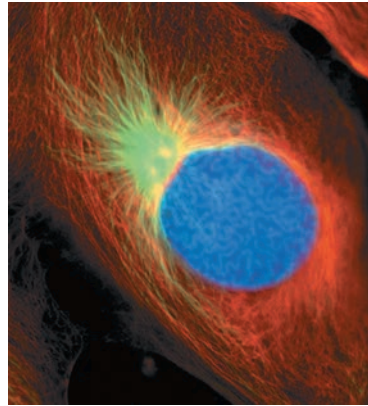
The Mitotic Spindle: A Closer Look

Many of the events of mitosis depend on the **mitotic spindle**, which begins to form in the cytoplasm during prophase. This structure consists of fibers made of microtubules and associated

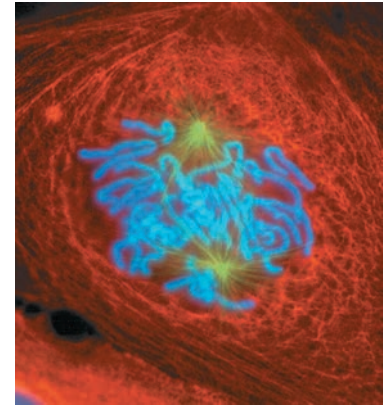
▼ Figure 12.7 Exploring Mitosis in an Animal Cell



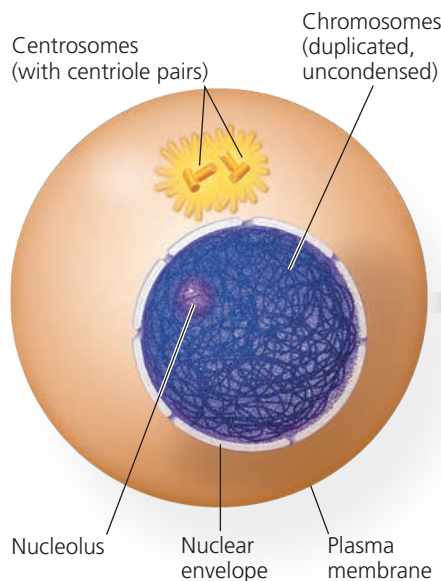
G₂ of Interphase



Prophase



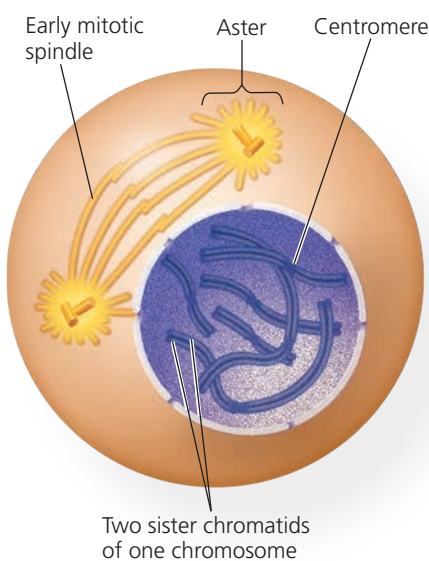
Prometaphase



G₂ of Interphase

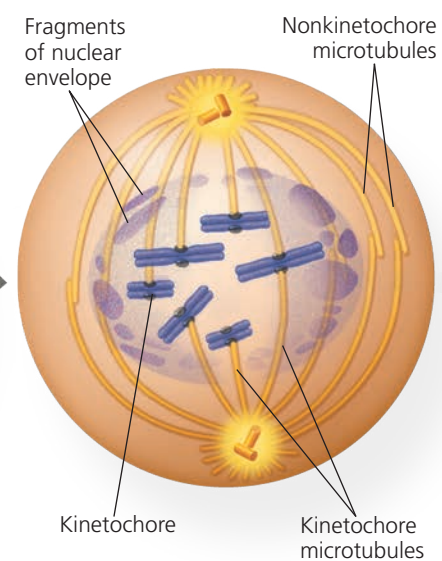
- A nuclear envelope encloses the nucleus.
- The nucleus contains one or more nucleoli (singular, *nucleolus*).
- Two centrosomes have formed by duplication of a single centrosome. Centrosomes are regions in animal cells that organize the microtubules of the spindle. Each centrosome contains two centrioles.
- Chromosomes, duplicated during S phase, cannot be seen individually because they have not yet condensed.

The fluorescence micrographs show dividing lung cells from a newt; this species has 22 chromosomes. Chromosomes appear blue, microtubules green, and intermediate filaments red. For simplicity, the drawings show only 6 chromosomes.



Prophase

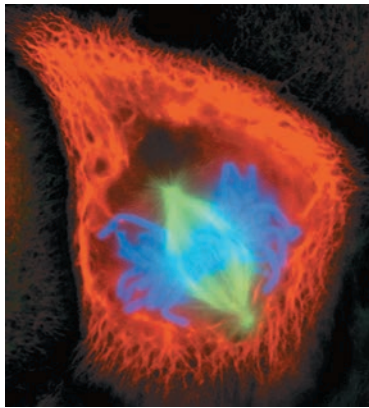
- The chromatin fibers become more tightly coiled, condensing into discrete chromosomes observable with a light microscope.
- The nucleoli disappear.
- Each duplicated chromosome appears as two identical sister chromatids joined at their centromeres and, in some species, all along their arms by cohesins (sister chromatid cohesion).
- The mitotic spindle (named for its shape) begins to form. It is composed of the centrosomes and the microtubules that extend from them. The radial arrays of shorter microtubules that extend from the centrosomes are called asters ("stars").
- The centrosomes move away from each other, propelled partly by the lengthening microtubules between them.



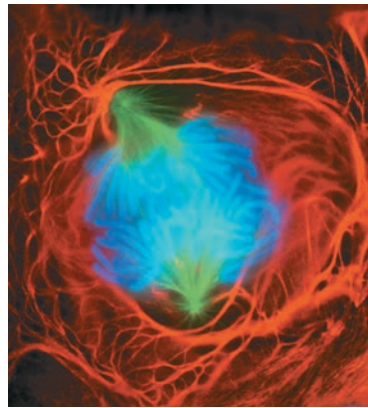
Prometaphase

- The nuclear envelope fragments.
- The microtubules extending from each centrosome can now invade the nuclear area.
- The centrosomes have become even more condensed.
- A kinetochore, a specialized protein structure, has now formed at the centromere of each chromatid (thus, two per chromosome).
- Some of the microtubules attach to the kinetochores, becoming "kinetochore microtubules," which jerk the chromosomes back and forth.
- Nonkinetochore microtubules interact with those from the opposite pole of the spindle, lengthening the cell.

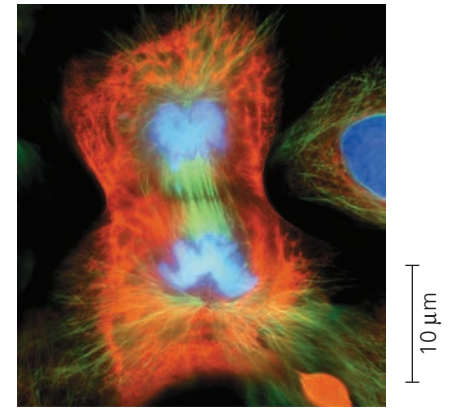
? How many molecules of DNA are in the prometaphase drawing? How many molecules per chromosome? How many double helices are there per chromosome? Per chromatid?



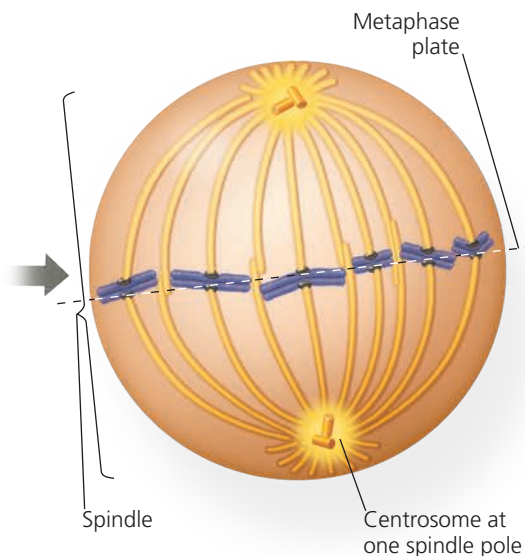
Metaphase



Anaphase

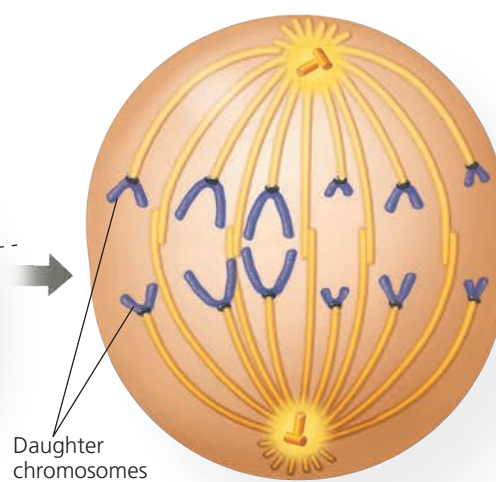


Telophase and Cytokinesis



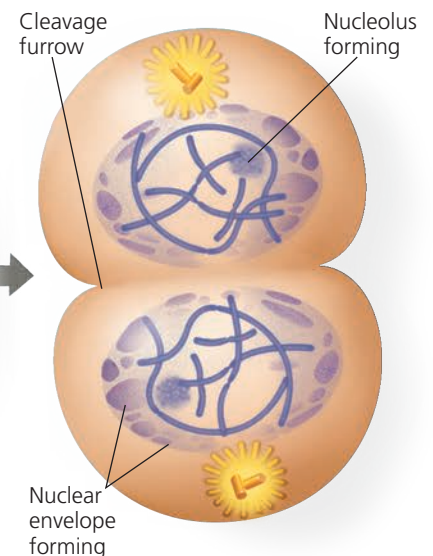
Metaphase

- The centrosomes are now at opposite poles of the cell.
- The chromosomes have all arrived at the *metaphase plate*, a plane that is equidistant between the spindle's two poles. The chromosomes' centromeres lie at the metaphase plate.
- For each chromosome, the kinetochores of the sister chromatids are attached to kinetochore microtubules coming from opposite poles.



Anaphase

- Anaphase is the shortest stage of mitosis, often lasting only a few minutes.
- Anaphase begins when the cohesin proteins are cleaved. This allows the two sister chromatids of each pair to part suddenly. Each chromatid thus becomes an independent chromosome.
- The two new daughter chromosomes begin moving toward opposite ends of the cell as their kinetochore microtubules shorten. Because these microtubules are attached at the centromere region, the centromeres are pulled ahead of the arms, moving at a rate of about 1 $\mu\text{m}/\text{min}$.
- The cell elongates as the nonkinetochore microtubules lengthen.
- By the end of anaphase, the two ends of the cell have equivalent—and complete—collections of chromosomes.



Telophase

- Two daughter nuclei form in the cell. Nuclear envelopes arise from the fragments of the parent cell's nuclear envelope and other portions of the endomembrane system.
- Nucleoli reappear.
- The chromosomes become less condensed.
- Any remaining spindle microtubules are depolymerized.
- Mitosis, the division of one nucleus into two genetically identical nuclei, is now complete.

Cytokinesis

- The division of the cytoplasm is usually well under way by late telophase, so the two daughter cells appear shortly after the end of mitosis.
- In animal cells, cytokinesis involves the formation of a cleavage furrow, which pinches the cell in two.

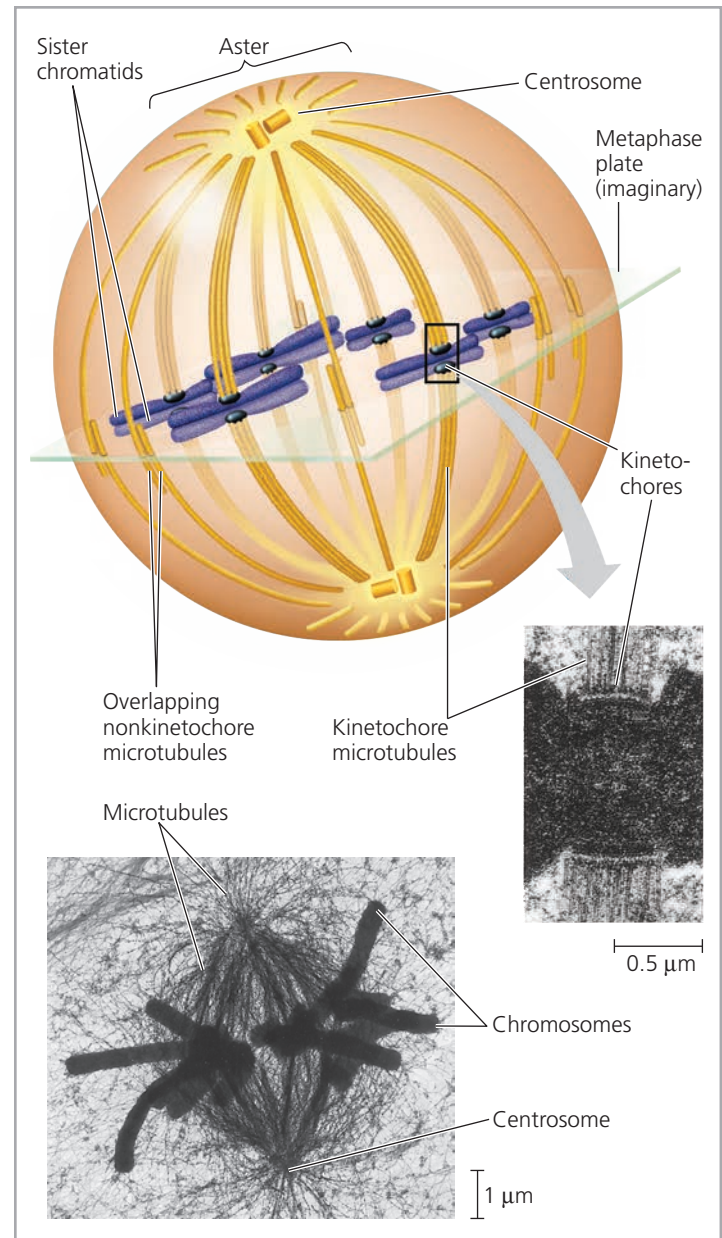
proteins. While the mitotic spindle assembles, the other microtubules of the cytoskeleton partially disassemble, providing the material used to construct the spindle. The spindle microtubules elongate (polymerize) by incorporating more subunits of the protein tubulin (see Table 7.1) and shorten (depolymerize) by losing subunits.

In animal cells, the assembly of spindle microtubules starts at the **centrosome**, a subcellular region containing material that functions throughout the cell cycle to organize the cell's microtubules. (It is also a type of *microtubule-organizing center*.) A pair of centrioles is located at the center of the centrosome, but they are not essential for cell division: If the centrioles are destroyed with a laser microbeam, a spindle nevertheless forms during mitosis. In fact, centrioles are not even present in plant cells, which do form mitotic spindles.

During interphase in animal cells, the single centrosome duplicates, forming two centrosomes, which remain near the nucleus. The two centrosomes move apart during prophase and prometaphase of mitosis as spindle microtubules grow out from them. By the end of prometaphase, the two centrosomes, one at each pole of the spindle, are at opposite ends of the cell. An *aster*, a radial array of short microtubules, extends from each centrosome. The spindle includes the centrosomes, the spindle microtubules, and the asters.

Each of the two sister chromatids of a duplicated chromosome has a **kinetochore**, a structure made up of proteins that have assembled on specific sections of DNA at each centromere. The chromosome's two kinetochores face in opposite directions. During prometaphase, some of the spindle microtubules attach to the kinetochores; these are called *kinetochore microtubules*. (The number of microtubules attached to a kinetochore varies among species, from one microtubule in yeast cells to 40 or so in some mammalian cells.) When one of a chromosome's kinetochores is "captured" by microtubules, the chromosome begins to move toward the pole from which those microtubules extend. However, this movement comes to a halt as soon as microtubules from the opposite pole attach to the kinetochore on the other chromatid. What happens next is like a tug-of-war that ends in a draw. The chromosome moves first in one direction and then in the other, back and forth, finally settling midway between the two ends of the cell. At metaphase, the centromeres of all the duplicated chromosomes are on a plane midway between the spindle's two poles. This plane is called the **metaphase plate**, which is an imaginary plate rather than an actual cellular structure (**Figure 12.8**). Meanwhile, microtubules that do not attach to kinetochores have been elongating, and by metaphase they overlap and interact with other *nonkinetochore microtubules* from the opposite pole of the spindle. By metaphase, the microtubules of the asters have also grown and are in contact with the plasma membrane. The spindle is now complete.

▼ **Figure 12.8 The mitotic spindle at metaphase.** The kinetochores of each chromosome's two sister chromatids face in opposite directions. Here, each kinetochore is attached to a cluster of kinetochore microtubules extending from the nearest centrosome. Nonkinetochore microtubules overlap at the metaphase plate (TEMs).



DRAW IT ► On the lower micrograph, draw a line indicating the position of the metaphase plate. Circle an aster. Draw arrows indicating the directions of chromosome movement once anaphase begins.



Video: Spindle Formation During Mitosis
Animation: Mitosis

The structure of the spindle correlates well with its function during anaphase. Anaphase begins suddenly when the cohesins holding together the sister chromatids of each chromosome are cleaved by an enzyme called *separase*. Once separated, the chromatids become individual chromosomes that move toward opposite ends of the cell.

How do the kinetochore microtubules function in this poleward movement of chromosomes? Apparently, two mechanisms are in play, both involving motor proteins. (To review how motor proteins move an object along a microtubule, see Figure 7.21.) Results of a cleverly designed experiment suggested that motor proteins on the kinetochores “walk” the chromosomes along the microtubules, which depolymerize at their kinetochore ends after the motor proteins have passed (**Figure 12.9**). (This is referred to as the “Pac-man” mechanism because of its resemblance to the arcade game character that moves by eating all the dots in its path.) However, other researchers, working with different cell types or cells from other species, have shown that chromosomes are “reeled in” by motor proteins at the spindle poles and that the microtubules depolymerize after they pass by these motor proteins at the poles. The general consensus now is that both mechanisms are used and that their relative contributions vary among cell types.

In a dividing animal cell, the nonkinetochore microtubules are responsible for elongating the whole cell during anaphase. Nonkinetochore microtubules from opposite poles overlap each other extensively during metaphase (see Figure 12.8). During anaphase, the region of overlap is reduced as motor proteins attached to the microtubules walk them away from one another, using energy from ATP. As the microtubules push apart from each other, their spindle poles are pushed apart, elongating the cell. At the same time, the microtubules lengthen somewhat by the addition of tubulin subunits to their overlapping ends. As a result, the microtubules continue to overlap.

At the end of anaphase, duplicate groups of chromosomes have arrived at opposite ends of the elongated parent cell. Nuclei re-form during telophase. Cytokinesis generally begins during anaphase or telophase, and the spindle eventually disassembles by depolymerization of microtubules.

Cytokinesis: A Closer Look

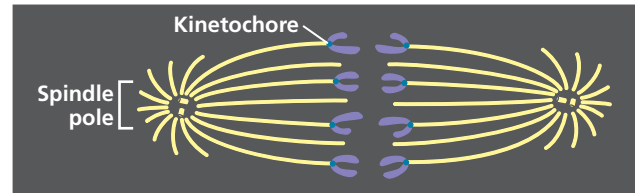
In animal cells, cytokinesis occurs by a process known as **cleavage**. The first sign of cleavage is the appearance of a **cleavage furrow**, a shallow groove in the cell surface near the old metaphase plate (**Figure 12.10a**). On the cytoplasmic side of the furrow is a contractile ring of actin microfilaments associated with molecules of the protein myosin. The actin microfilaments interact with the myosin molecules, causing the ring to contract. The contraction of the dividing cell’s ring of microfilaments is like the pulling of a drawstring. The cleavage furrow deepens until the parent cell is pinched in two, producing two completely separated cells, each with its own nucleus and its own share of cytosol, organelles, and other subcellular structures.

Cytokinesis in plant cells, which have cell walls, is markedly different. There is no cleavage furrow. Instead, during telophase, vesicles derived from the Golgi apparatus move along microtubules to the middle of the cell, where they coalesce, producing a **cell plate**. Cell wall materials

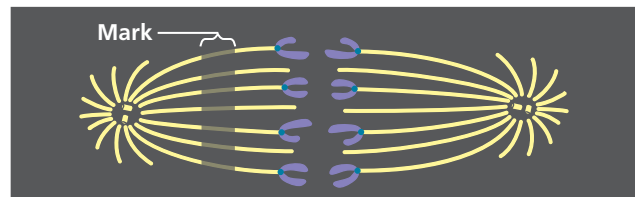
▼ Figure 12.9

Inquiry At which end do kinetochore microtubules shorten during anaphase?

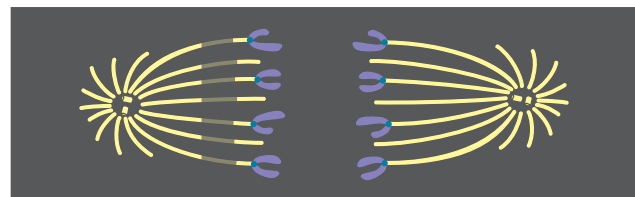
Experiment Gary Borisy and colleagues at the University of Wisconsin wanted to determine whether kinetochore microtubules depolymerize at the kinetochore end or the pole end as chromosomes move toward the poles during mitosis. First they labeled the microtubules of a pig kidney cell in early anaphase with a yellow fluorescent dye. (Nonkinetochore microtubules are not shown.)



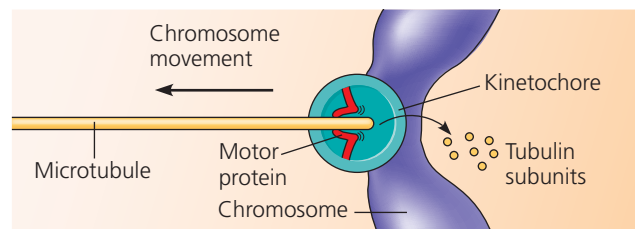
Then they marked a region of the kinetochore microtubules between one spindle pole and the chromosomes by using a laser to eliminate the fluorescence from that region, while leaving the microtubules intact (see below). As anaphase proceeded, they monitored the changes in microtubule length on either side of the mark.



Results As the chromosomes moved poleward, the microtubule segments on the kinetochore side of the mark shortened, while those on the spindle pole side stayed the same length.



Conclusion During anaphase in this cell type, chromosome movement is correlated with kinetochore microtubules shortening at their kinetochore ends and not at their spindle pole ends. This experiment supports the hypothesis that during anaphase, a chromosome is walked along a microtubule as the microtubule depolymerizes at its kinetochore end, releasing tubulin subunits.



Data from G. J. Gorbsky, P. J. Sammak, and G. G. Borisy, Chromosomes move poleward in anaphase along stationary microtubules that coordinately disassemble from their kinetochore ends, *Journal of Cell Biology* 104:9–18 (1987).

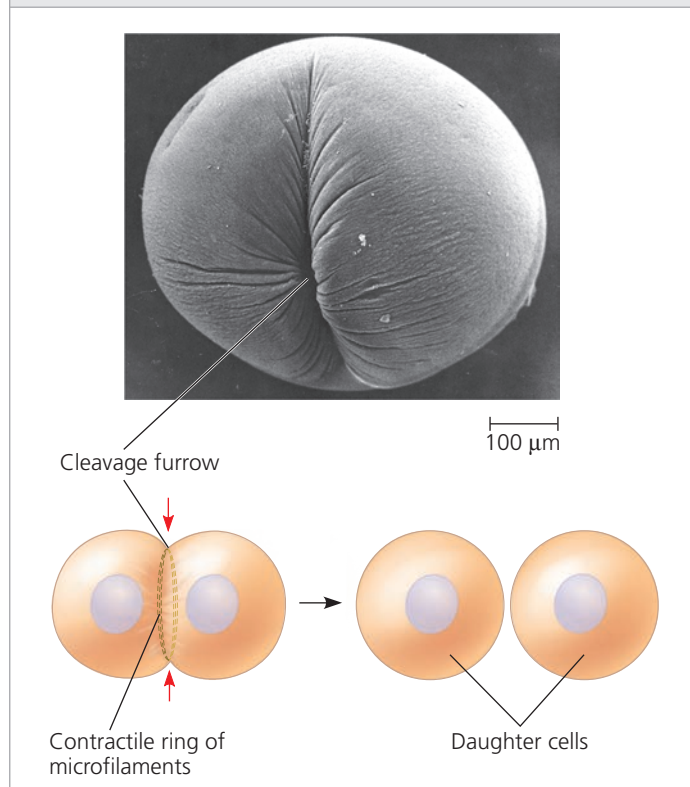
WHAT IF? > If this experiment had been done on a cell type in which “reeling in” at the poles was the main cause of chromosome movement, how would the mark have moved relative to the poles? How would the microtubule portions on either side of the mark have changed?



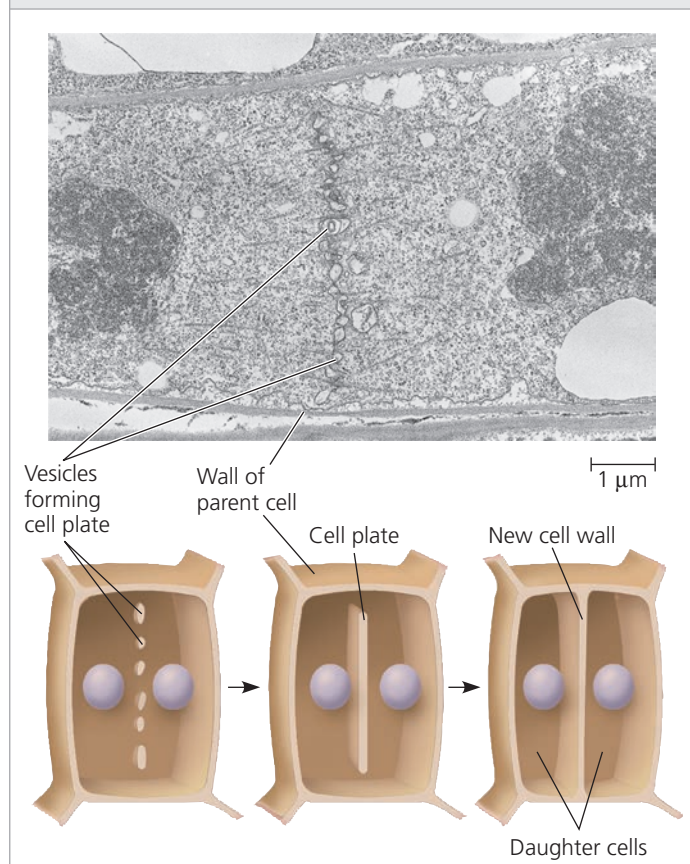
Animation: Microtubule Depolymerization

▼ **Figure 12.10 Cytokinesis in animal and plant cells.**

(a) Cleavage of an animal cell (SEM)



(b) Cell plate formation in a plant cell (TEM)



 **Animation: Cytokinesis**
Video: Cytokinesis in an Animal Cell

carried in the vesicles collect inside the cell plate as it grows (**Figure 12.10b**). The cell plate enlarges until its surrounding membrane fuses with the plasma membrane along the perimeter of the cell. Two daughter cells result, each with its own plasma membrane. Meanwhile, a new cell wall arising from the contents of the cell plate forms between the daughter cells.

Figure 12.11 is a series of micrographs of a dividing plant cell. Examining this figure will help you review mitosis and cytokinesis.

Binary Fission in Bacteria

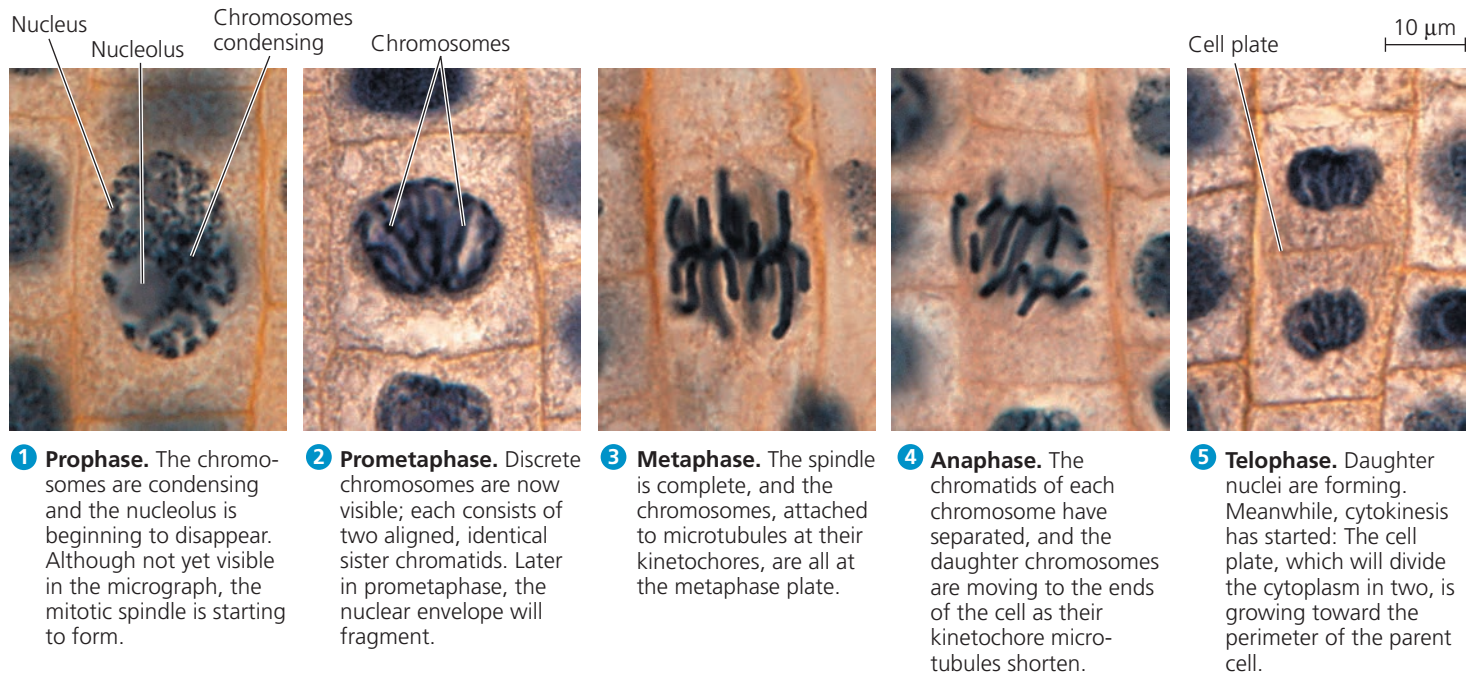
Prokaryotes (bacteria and archaea) can undergo a type of reproduction in which the cell grows to roughly double its size and then divides to form two cells. The term **binary fission**, meaning “division in half,” refers to this process and to the asexual reproduction of single-celled eukaryotes, such as the amoeba in **Figure 12.2a**. However, the process in eukaryotes involves mitosis, while that in prokaryotes does not.

In bacteria, most genes are carried on a single bacterial chromosome that consists of a circular DNA molecule and associated proteins. Although bacteria are smaller and simpler than eukaryotic cells, the challenge of replicating their genomes in an orderly fashion and distributing the copies equally to two daughter cells is still formidable. For example, when it is fully stretched out, the chromosome of the bacterium *Escherichia coli* is about 500 times as long as the cell. For such a long chromosome to fit within the cell, it must be highly coiled and folded.

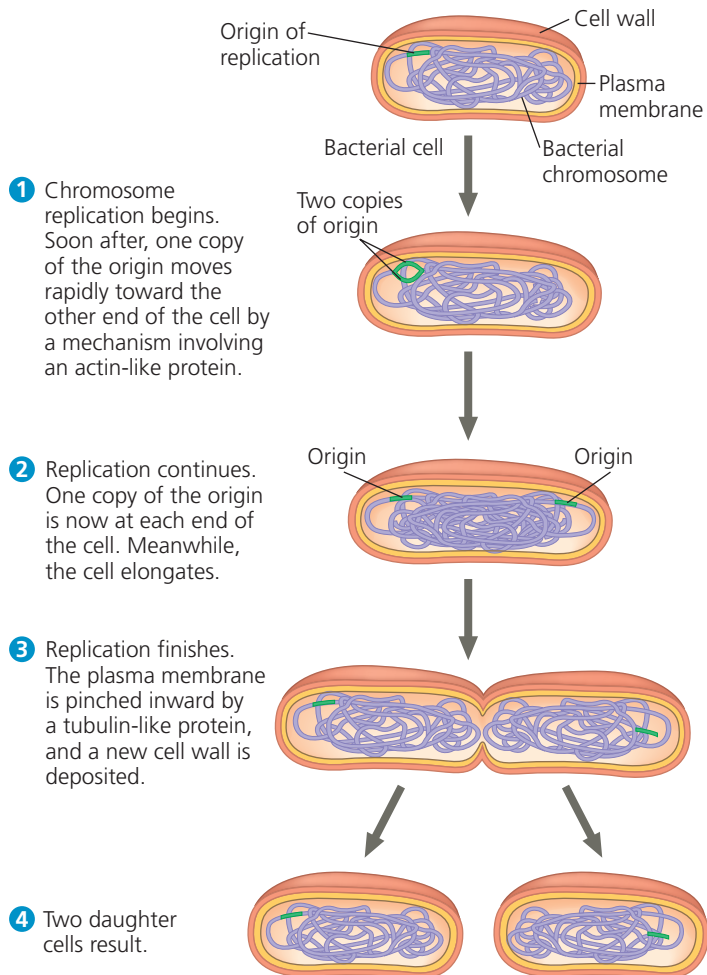
In some bacteria, the process of cell division is initiated when the DNA of the bacterial chromosome begins to replicate at a specific place on the chromosome called the **origin of replication**, producing two origins. As the chromosome continues to replicate, one origin moves rapidly toward the opposite end of the cell (**Figure 12.12**). While the chromosome is replicating, the cell elongates. When replication is complete and the bacterium has reached about twice its initial size, proteins cause its plasma membrane to pinch inward, dividing the parent bacterial cell into two daughter cells. In this way, each cell inherits a complete genome.

Using the techniques of modern DNA technology to tag the origins of replication with molecules that glow green in fluorescence microscopy (see **Figure 7.3**), researchers have directly observed the movement of bacterial chromosomes. This movement is reminiscent of the poleward movements of the centromere regions of eukaryotic chromosomes during anaphase of mitosis, but bacteria don’t have visible mitotic spindles or even microtubules. In most bacterial species studied, the two origins of replication end up at opposite ends of the cell or in some other very specific location, possibly anchored there by one or more proteins. How bacterial chromosomes move and how their specific location is established and maintained are active areas of research. Several proteins that play important roles have been identified. Polymerization of one protein resembling eukaryotic actin

▼ **Figure 12.11 Mitosis in a plant cell.** These light micrographs show mitosis in cells of an onion root.



▼ **Figure 12.12 Bacterial cell division by binary fission.** The bacterium shown here has a single, circular chromosome.



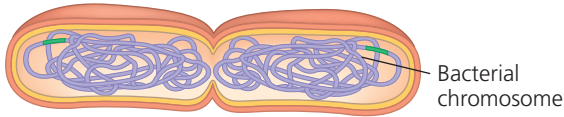
apparently functions in bacterial chromosome movement during cell division, and another protein that is related to tubulin helps pinch the plasma membrane inward, separating the two bacterial daughter cells.

The Evolution of Mitosis

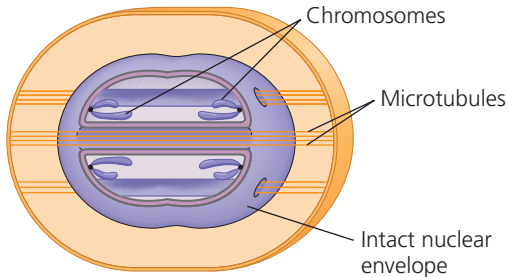
EVOLUTION Given that prokaryotes preceded eukaryotes on Earth by more than a billion years, we might hypothesize that mitosis evolved from simpler prokaryotic mechanisms of cell reproduction. The fact that some of the proteins involved in bacterial binary fission are related to eukaryotic proteins that function in mitosis supports that hypothesis.

As eukaryotes with nuclear envelopes and larger genomes evolved, the ancestral process of binary fission, seen today in bacteria, somehow gave rise to mitosis. Variations on cell division exist in different groups of organisms. These variant processes may be similar to mechanisms used by ancestral species and thus may resemble steps in the evolution of mitosis from a binary fission-like process presumably carried out by very early bacteria. Possible intermediate stages are suggested by two unusual types of nuclear division found today in certain unicellular eukaryotes—dinoflagellates, diatoms, and some yeasts (**Figure 12.13**). These two modes of nuclear division are thought to be cases where ancestral mechanisms have remained relatively unchanged over evolutionary time. In both types, the nuclear envelope remains intact, in contrast to what happens in most eukaryotic cells. Keep in mind, however, that we can't observe cell division in cells of extinct species. This hypothesis uses only currently existing species as examples and must ignore any potential intermediate mechanisms used by species that disappeared long ago.

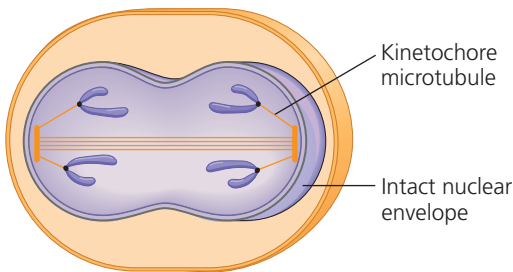
▼ **Figure 12.13 Mechanisms of cell division in several groups of organisms.** Some unicellular eukaryotes existing today have mechanisms of cell division that may resemble intermediate steps in the evolution of mitosis. Except for (a), cell walls are not shown.



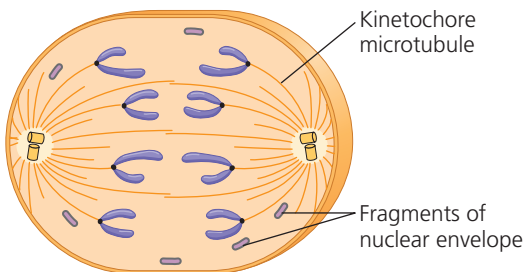
(a) Bacteria. During binary fission in bacteria, the origins of the daughter chromosomes move to opposite ends of the cell. The mechanism involves polymerization of actin-like molecules, and possibly proteins that may anchor the daughter chromosomes to specific sites on the plasma membrane.



(b) Dinoflagellates. In unicellular protists called dinoflagellates, the chromosomes attach to the nuclear envelope, which remains intact during cell division. Microtubules pass through the nucleus inside cytoplasmic tunnels, reinforcing the spatial orientation of the nucleus, which then divides in a process reminiscent of bacterial binary fission.



(c) Diatoms and some yeasts. In these two other groups of unicellular eukaryotes, the nuclear envelope also remains intact during cell division. In these organisms, the microtubules form a spindle *within* the nucleus. Microtubules separate the chromosomes, and the nucleus splits into two daughter nuclei.



(d) Most eukaryotes. In most other eukaryotes, including plants and animals, the spindle forms outside the nucleus, and the nuclear envelope breaks down during mitosis. Microtubules separate the chromosomes, and two nuclear envelopes then form.



Video: Nuclear Envelope Breakdown and Formation During Mitosis in *C. elegans*, a Eukaryote

CONCEPT CHECK 12.2

1. How many chromosomes are shown in the illustration in Figure 12.8? Are they duplicated? How many chromatids are shown?
2. Compare cytokinesis in animal cells and plant cells.
3. During which stage of the cell cycle does each of the sister chromatids become an independent chromosome?
4. Compare the roles of tubulin and actin during eukaryotic cell division with the roles of tubulin-like and actin-like proteins during bacterial binary fission.
5. A kinetochore has been compared to a coupling device that connects a motor to the cargo that it moves. Explain.
6. **MAKE CONNECTIONS** ► What other functions do actin and tubulin carry out? Name the proteins they interact with to do so. (Review Figures 7.21a and 7.26a.)

For suggested answers, see Appendix A.

CONCEPT 12.3

The eukaryotic cell cycle is regulated by a molecular control system

The timing and rate of cell division in different parts of a plant or animal are crucial to normal growth, development, and maintenance. The frequency of cell division varies with the type of cell. For example, human skin cells divide frequently throughout life, whereas liver cells maintain the ability to divide but keep it in reserve until an appropriate need arises—say, to repair a wound. Some of the most specialized cells, such as fully formed nerve cells and muscle cells, do not divide at all in a mature human. These cell cycle differences result from regulation at the molecular level. The mechanisms of this regulation are of great interest, not only to understand the life cycles of normal cells but also to learn how cancer cells manage to escape the usual controls.

The Cell Cycle Control System

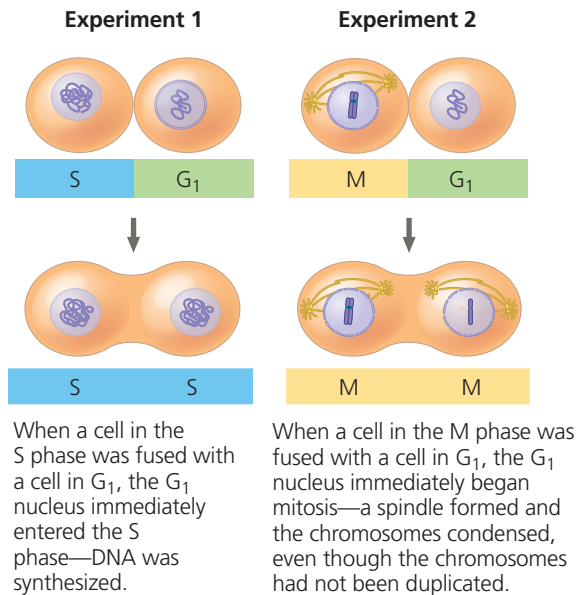
What controls the cell cycle? In the early 1970s, a variety of experiments led to the hypothesis that the cell cycle is driven by specific signaling molecules present in the cytoplasm. Some of the first strong evidence for this hypothesis came from experiments with mammalian cells grown in culture. In these experiments, two cells in different phases of the cell cycle were fused to form a single cell with two nuclei (**Figure 12.14**). If one of the original cells was in the S phase and the other was in G_1 , the G_1 nucleus immediately entered the S phase, as though stimulated by signaling molecules present in the cytoplasm of the first cell. Similarly, if a cell undergoing mitosis (M phase) was fused with another cell in any stage of its cell cycle, even G_1 , the second nucleus immediately entered mitosis, with condensation of the chromatin and formation of a mitotic spindle.

The experiment shown in Figure 12.14 and other experiments on animal cells and yeasts demonstrated that the sequential events of the cell cycle are directed by a distinct **cell cycle control system**, a cyclically operating set of

▼ Figure 12.14

Inquiry Do molecular signals in the cytoplasm regulate the cell cycle?

Experiment Researchers at the University of Colorado wondered whether a cell's progression through the cell cycle is controlled by cytoplasmic molecules. They induced cultured mammalian cells at different phases of the cell cycle to fuse. Two experiments are shown.



Conclusion The results of fusing a G₁ cell with a cell in the S or M phase of the cell cycle suggest that molecules present in the cytoplasm during the S or M phase control the progression to those phases.

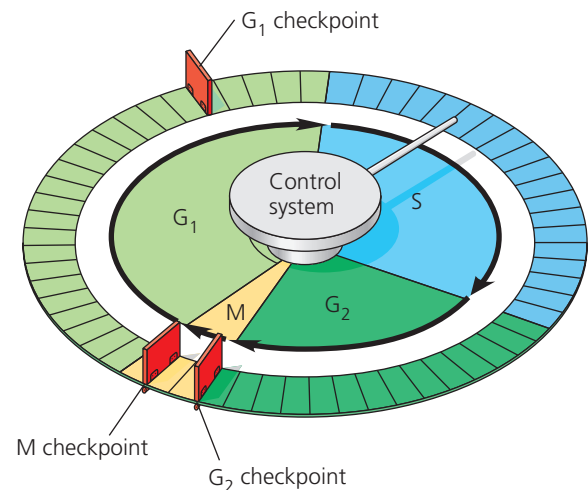
Data from R. T. Johnson and P. N. Rao, Mammalian cell fusion: Induction of premature chromosome condensation in interphase nuclei, *Nature* 226:717–722 (1970).

WHAT IF? > If the progression of phases did not depend on cytoplasmic molecules and, instead, each phase automatically began when the previous one was complete, how would the results have differed?

molecules in the cell that both triggers and coordinates key events in the cell cycle (**Figure 12.15**). The cell cycle control system has been compared to the control device of a washing machine. Like the washer's timing device, the cell cycle control system proceeds on its own, according to a built-in clock. However, just as a washer's cycle is subject to both internal control (such as the sensor that detects when the tub is filled with water) and external adjustment (such as starting or stopping the machine), the cell cycle is regulated at certain checkpoints by both internal and external signals. A **checkpoint** in the cell cycle is a control point where stop and go-ahead signals can regulate the cycle. Three important checkpoints are found in the G₁, G₂, and M phases (red gates in Figure 12.15), which will be discussed shortly.

To understand how cell cycle checkpoints work, we'll first identify some of the molecules that make up the cell cycle control system (the molecular basis for the cell cycle clock) and describe how a cell progresses through

▼ Figure 12.15 Mechanical analogy for the cell cycle control system. In this diagram, the flat “stepping stones” around the perimeter represent sequential events. Like the control device of a washing machine, the cell cycle control system proceeds on its own, driven by a built-in clock. However, the system is subject to internal and external regulation at various checkpoints; three important checkpoints are shown (red).



Animation: Control of the Cell Cycle

the cycle. We'll then consider the internal and external checkpoint signals that can make the clock either pause or continue.

The Cell Cycle Clock: Cyclins and Cyclin-Dependent Kinases

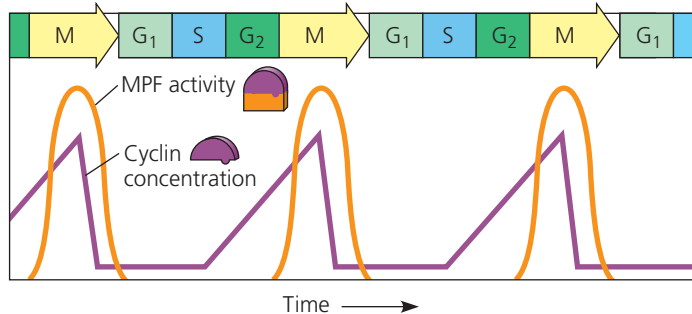
Rhythmic fluctuations in the abundance and activity of cell cycle control molecules pace the sequential events of the cell cycle. These regulatory molecules are mainly proteins of two types: protein kinases and cyclins. Protein kinases are enzymes that activate or inactivate other proteins by phosphorylating them (see Concept 9.3).

Many of the kinases that drive the cell cycle are actually present at a constant concentration in the growing cell, but much of the time they are in an inactive form. To be active, such a kinase must be attached to a **cyclin**, a protein that gets its name from its cyclically fluctuating concentration in the cell. Because of this requirement, these kinases are called **cyclin-dependent kinases**, or **Cdks**. The activity of a Cdk rises and falls with changes in the concentration of its cyclin partner. **Figure 12.16a** shows the fluctuating activity of **MPF**, the cyclin-Cdk complex that was discovered first (in frog eggs). Note that the peaks of MPF activity correspond to the peaks of cyclin concentration. The cyclin level rises during the S and G₂ phases and then falls abruptly during M phase.

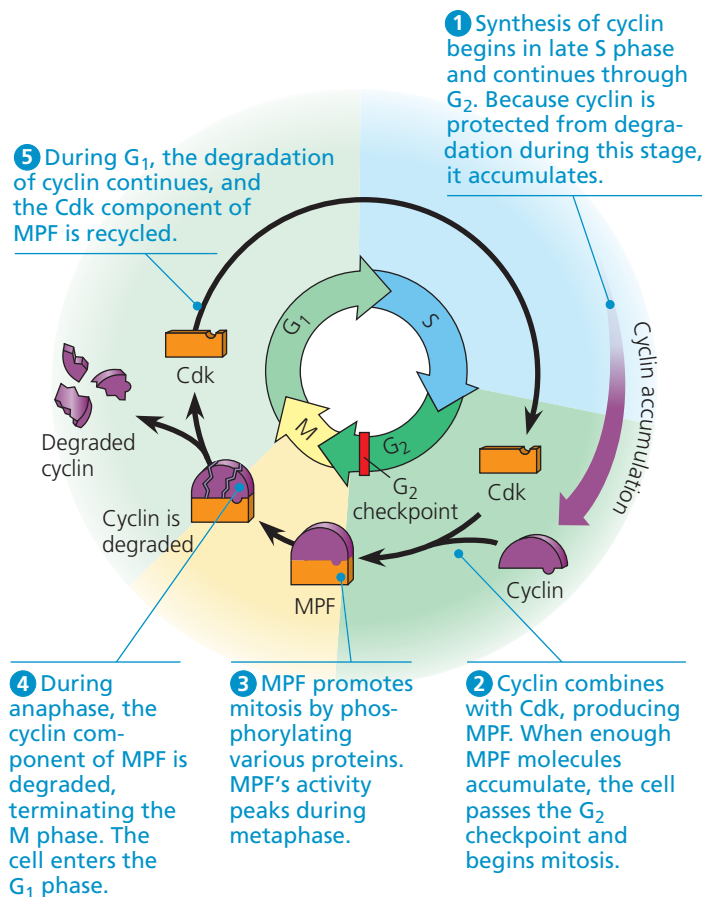
The initials MPF stand for “maturation-promoting factor,” but we can think of MPF as “M-phase-promoting factor” because it triggers the cell's passage into the M phase, past the G₂ checkpoint. When cyclins that accumulate during G₂ associate with Cdk molecules, the resulting MPF complex is active—it phosphorylates a variety of proteins, initiating

mitosis (**Figure 12.16b**). MPF acts both directly as a kinase and indirectly by activating other kinases. For example, MPF causes phosphorylation of various proteins of the nuclear lamina (see Figure 7.9), which promotes fragmentation of the nuclear envelope during prometaphase of mitosis. There is also evidence that MPF contributes to molecular events required for chromosome condensation and spindle formation during prophase.

Figure 12.16 Molecular control of the cell cycle at the G₂ checkpoint. The steps of the cell cycle are timed by rhythmic fluctuations in the activity of cyclin-dependent kinases (Cdks). Here we focus on a cyclin-Cdk complex in animal cells called MPF, which acts at the G₂ checkpoint as a go-ahead signal, triggering the events of mitosis.



(a) Fluctuation of MPF activity and cyclin concentration during the cell cycle



(b) Molecular mechanisms that help regulate the cell cycle

VISUAL SKILLS Explain how the events in the diagram in (b) are related to the "Time" axis of the graph in (a), beginning at the left.

During anaphase, MPF helps switch itself off by initiating a process that leads to the destruction of its own cyclin. The noncyclin part of MPF, the Cdk, persists in the cell, inactive until it becomes part of MPF again by associating with new cyclin molecules synthesized during the S and G₂ phases of the next round of the cycle.

The fluctuating activities of different cyclin-Cdk complexes are of major importance in controlling all the stages of the cell cycle; they also give the go-ahead signals at some checkpoints. As mentioned above, MPF controls the cell's passage through the G₂ checkpoint. Cell behavior at the G₁ checkpoint is also regulated by the activity of cyclin-Cdk protein complexes. Animal cells appear to have at least three Cdk proteins and several different cyclins that operate at this checkpoint. Next, let's consider checkpoints in more detail.

Stop and Go Signs: Internal and External Signals at the Checkpoints

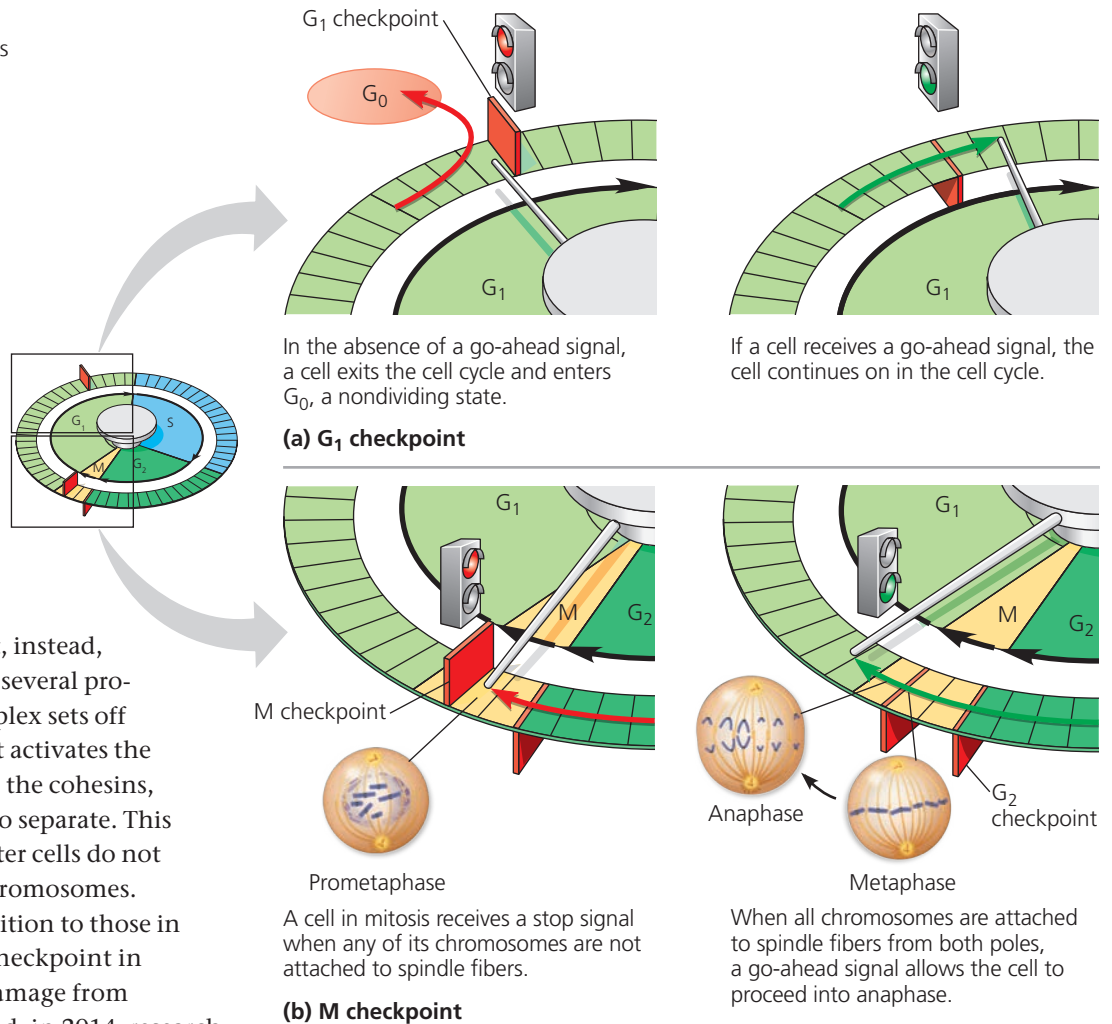
Animal cells generally have built-in stop signals that halt the cell cycle at checkpoints until overridden by go-ahead signals. (The signals are transmitted within the cell by the kinds of signal transduction pathways discussed in Chapter 9.) Many signals registered at checkpoints come from cellular surveillance mechanisms inside the cell. These signals report whether crucial cellular processes that should have occurred by that point have in fact been completed correctly and thus whether or not the cell cycle should proceed. Checkpoints also register signals from outside the cell.

Three important checkpoints are those in the G₁, G₂, and M phases (see Figure 12.15). For many cells, the G₁ checkpoint seems to be the most important. If a cell receives a go-ahead signal at the G₁ checkpoint, it will usually complete the G₁, S, G₂, and M phases and divide. If it does not receive a go-ahead signal at that point, it may exit the cycle, switching into a nondividing state called the **G₀ phase** (**Figure 12.17a**). Most cells of the human body are actually in the G₀ phase. As mentioned earlier, mature nerve cells and muscle cells never divide. Other cells, such as liver cells, can be "called back" from the G₀ phase to the cell cycle by external cues, such as growth factors released during injury.

Biologists are currently working out the pathways that link signals originating inside and outside the cell with the responses by cyclin-dependent kinases and other proteins. An example of an internal signal occurs at the third important checkpoint, the M checkpoint (**Figure 12.17b**). Anaphase, the separation of sister chromatids, does not begin until all the chromosomes are properly attached to the spindle at the metaphase plate. Researchers have learned that as long as some kinetochores are unattached to spindle microtubules, the sister chromatids remain together, delaying anaphase. Only when the kinetochores of all the chromosomes are properly attached to the spindle does the appropriate regulatory protein complex become activated. (In this case, the regulatory molecule

➤ **Figure 12.17 Two important checkpoints.** At certain checkpoints in the cell cycle (red gates), cells do different things depending on the signals they receive. Events of the (a) G₁ and (b) M checkpoints are shown. In (b), the G₂ checkpoint has already been passed by the cell.

WHAT IF? ➤ What might be the result if the cell ignored either checkpoint and progressed through the cell cycle?



is not a cyclin-Cdk complex but, instead, a different complex made up of several proteins.) Once activated, the complex sets off a chain of molecular events that activates the enzyme separase, which cleaves the cohesins, allowing the sister chromatids to separate. This mechanism ensures that daughter cells do not end up with missing or extra chromosomes.

There are checkpoints in addition to those in G₁, G₂, and M. For instance, a checkpoint in S phase stops cells with DNA damage from proceeding in the cell cycle. And, in 2014, researchers presented evidence for another checkpoint between anaphase and telophase that ensures anaphase is completed and the chromosomes are well separated before cytokinesis can begin, thus avoiding chromosomal damage.

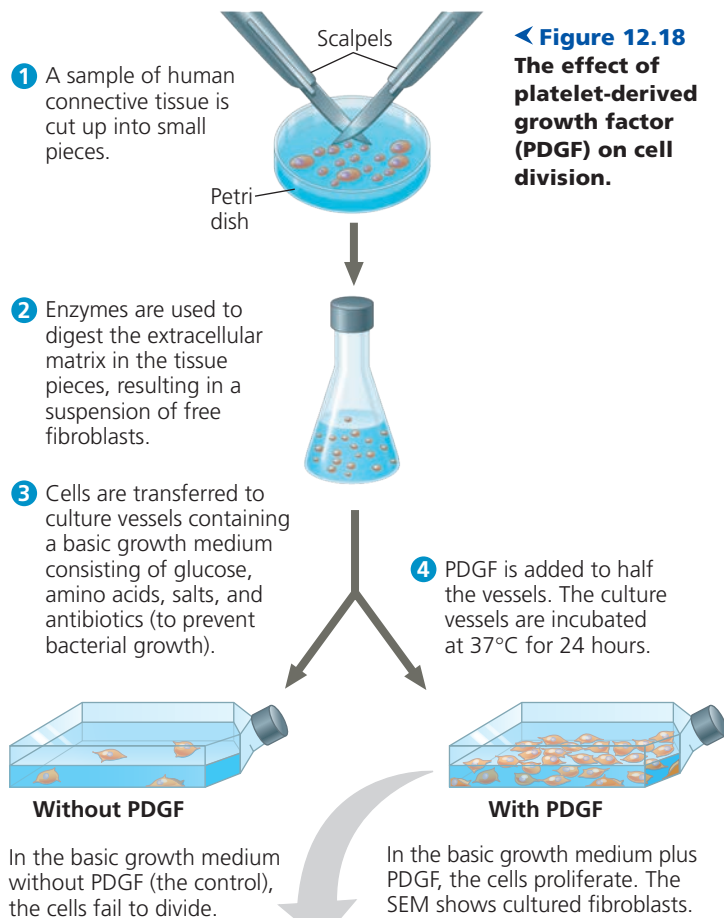
What about the stop and go-ahead signals themselves—what are the signaling molecules? Studies using animal cells in culture have led to the identification of many external factors, both chemical and physical, that can influence cell division. For example, cells fail to divide if an essential nutrient is lacking in the culture medium. (This is analogous to trying to run a washing machine without the water supply hooked up; an internal sensor won't allow the machine to continue past the point where water is needed.) And even if all other conditions are favorable, most types of mammalian cells divide in culture only if the growth medium includes specific growth factors. As mentioned in Concept 9.1, a **growth factor** is a protein released by certain cells that stimulates other cells to divide. Different cell types respond specifically to different growth factors or combinations of growth factors.

Consider, for example, *platelet-derived growth factor (PDGF)*, which is made by blood cell fragments called platelets. The experiment illustrated in **Figure 12.18** demonstrates that PDGF is required for the division of cultured fibroblasts, a type of connective tissue cell. Fibroblasts have PDGF

receptors on their plasma membranes. The binding of PDGF molecules to these receptors (which are receptor tyrosine kinases; see Figure 9.8) triggers a signal transduction pathway that allows the cells to pass the G₁ checkpoint and divide. PDGF stimulates fibroblast division not only in the artificial conditions of cell culture but also in an animal's body. When an injury occurs, platelets release PDGF in the vicinity. The resulting proliferation of fibroblasts helps heal the wound.

The effect of an external physical factor on cell division is clearly seen in **density-dependent inhibition**, a phenomenon in which crowded cells stop dividing (**Figure 12.19a**). As first observed many years ago, cultured cells normally divide until they form a single layer of cells on the inner surface of a culture flask, at which point the cells stop dividing. If some cells are removed, those bordering the open space begin dividing again and continue until the vacancy is filled. Follow-up studies revealed that the binding of a cell-surface protein to its counterpart on an adjoining cell sends a signal to both cells that inhibits cell division, preventing them from moving forward in the cell cycle, even in the presence of growth factors.

Most animal cells also exhibit **anchorage dependence** (see Figure 12.19a). To divide, they must be attached to a substratum, such as the inside of a culture flask or the



MAKE CONNECTIONS >

PDGF signals cells by binding to a cell-surface receptor tyrosine kinase. If you added a chemical that blocked phosphorylation, how would the results differ? (See Figure 9.8.)

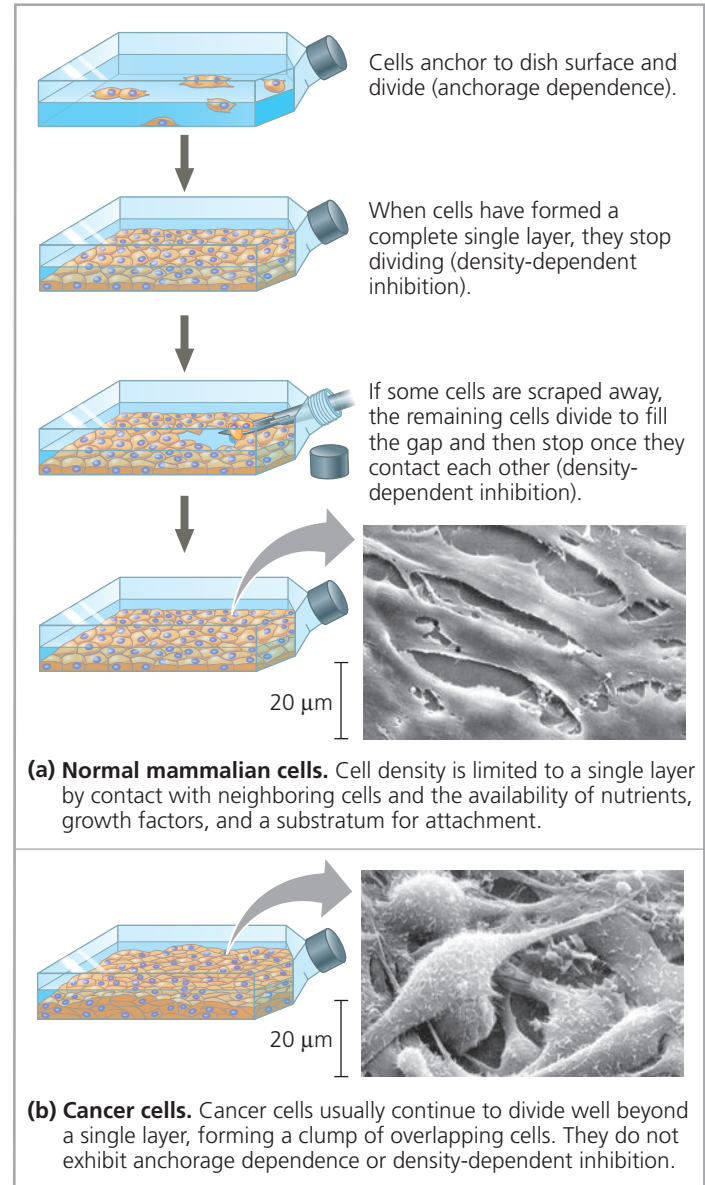
extracellular matrix of a tissue. Experiments suggest that like cell density, anchorage is signaled to the cell cycle control system via pathways involving plasma membrane proteins and elements of the cytoskeleton linked to them.

Density-dependent inhibition and anchorage dependence appear to function not only in cell culture but also in the body's tissues, checking the growth of cells at some optimal density and location during embryonic development and throughout an organism's life. Cancer cells, which we discuss next, exhibit neither density-dependent inhibition nor anchorage dependence (**Figure 12.19b**).

Loss of Cell Cycle Controls in Cancer Cells

Cancer cells do not heed the normal signals that regulate the cell cycle. In culture, they do not stop dividing when growth factors are depleted. A logical hypothesis is that cancer cells do not need growth factors in their culture medium to grow and divide. They may make a required

Figure 12.19 **Density-dependent inhibition and anchorage dependence of cell division.** Individual cells are shown disproportionately large in the drawings.



growth factor themselves, or they may have an abnormality in the signaling pathway that conveys the growth factor's signal to the cell cycle control system even in the absence of that factor. Another possibility is an abnormal cell cycle control system. In these scenarios, the underlying basis of the abnormality is almost always a change in one or more genes (for example, a mutation) that alters the function of their protein products, resulting in faulty cell cycle control.

There are other important differences between normal cells and cancer cells that reflect derangements of the cell cycle. If and when they stop dividing, cancer cells do so at random points in the cycle, rather than at the normal checkpoints. Moreover, cancer cells can go on dividing indefinitely

in culture if they are given a continual supply of nutrients; in essence, they are “immortal.” A striking example is a cell line that has been reproducing in culture since 1951. Cells of this line are called HeLa cells because their original source was a tumor removed from a woman named *Henrietta Lacks*. Cells in culture that acquire the ability to divide indefinitely are said to have undergone **transformation**, the process that causes them to behave like cancer cells. By contrast, nearly all normal, nontransformed mammalian cells growing in culture divide only about 20 to 50 times before they stop dividing, age, and die. Finally, cancer cells evade the normal controls that trigger a cell to undergo apoptosis when something is wrong—for example, when an irreparable mistake has occurred during DNA replication preceding mitosis.



ABC News Video: Henrietta Lacks' Cells

Abnormal cell behavior in the body can be catastrophic. The problem begins when a single cell in a tissue undergoes the first of many steps that converts a normal cell to a cancer cell. Such a cell often has altered proteins on its surface, and the body's immune system normally recognizes the cell as “nonself”—an insurgent—and destroys it. However, if the cell evades destruction, it may proliferate and form a tumor, a mass of abnormal cells within otherwise normal tissue. The abnormal cells may remain at the original site if they have too few genetic and cellular changes to survive at another site. In that case, the tumor is called a **benign tumor**. Most benign tumors do not cause serious problems (depending on their location) and can be removed by surgery. In contrast, a **malignant tumor** includes cells whose genetic and cellular changes enable them to spread to new tissues and impair the functions of one or more organs; these cells are also sometimes called *transformed* cells (although usage of this term is generally restricted to cells in culture). An individual with a malignant tumor is said to have cancer (**Figure 12.20**).

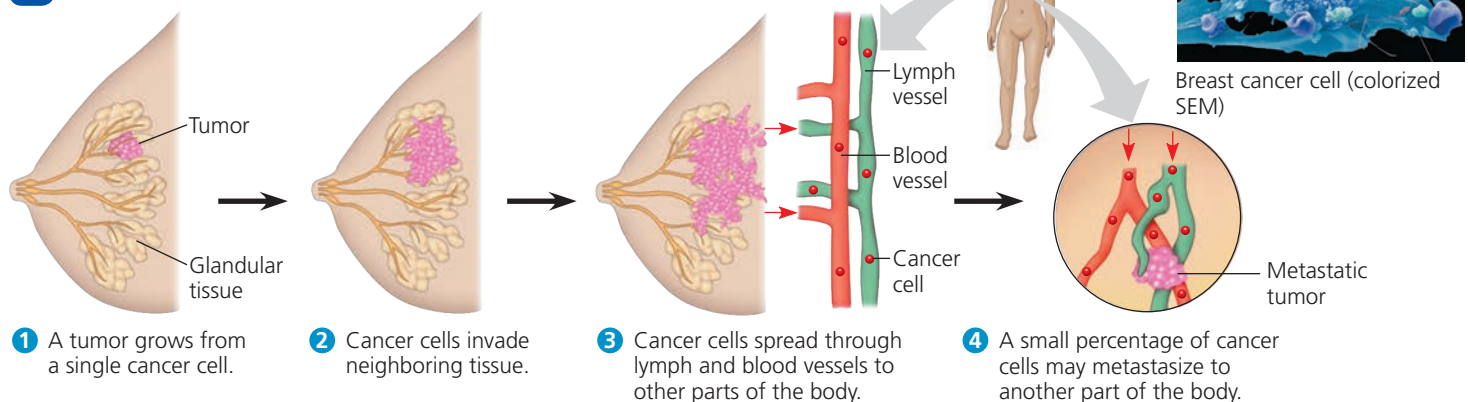
The changes that have occurred in cells of malignant tumors show up in many ways besides excessive proliferation. These cells may have unusual numbers of chromosomes, though whether this is a cause or an effect of tumor-related changes is a topic of debate. Their metabolism may be altered, and they may cease to function in any constructive way. Abnormal changes on the cell surface cause cancer cells to lose attachments to neighboring cells and the extracellular matrix, allowing them to spread into nearby tissues. Cancer cells may also secrete signaling molecules that cause blood vessels to grow toward the tumor. A few tumor cells may separate from the original tumor, enter blood vessels and lymph vessels, and travel to other parts of the body. There, they may proliferate and form a new tumor. This spread of cancer cells to locations distant from their original site is called **metastasis** (see Figure 12.20).

A tumor that appears to be localized may be treated with high-energy radiation, which damages DNA in cancer cells much more than DNA in normal cells, apparently because the majority of cancer cells have lost the ability to repair such damage. To treat known or suspected metastatic tumors, chemotherapy is used, in which drugs that are toxic to actively dividing cells are administered through the circulatory system. As you might expect, chemotherapeutic drugs interfere with specific steps in the cell cycle. For example, the drug Taxol freezes the mitotic spindle by preventing microtubule depolymerization, which stops actively dividing cells from proceeding past metaphase and leads to their destruction. The side effects of chemotherapy are due to the effects of the drugs on normal cells that divide frequently, due to the function of that cell type in the organism. For example, nausea results from chemotherapy's effects on intestinal cells, hair loss from effects on hair follicle cells, and susceptibility to infection from effects on immune system cells. You'll work with data from an experiment involving a potential chemotherapeutic agent in the **Scientific Skills Exercise**.

▼ **Figure 12.20** The growth and metastasis of a malignant breast tumor. A series of genetic and cellular changes contribute to a tumor becoming malignant (cancerous). The cells of malignant tumors grow in an uncontrolled way and can spread to neighboring tissues and, via lymph and blood vessels, to other parts of the body. The spread of cancer cells beyond their original site is called metastasis.



Video: Tumor Growth in a Living Mouse



SCIENTIFIC SKILLS EXERCISE

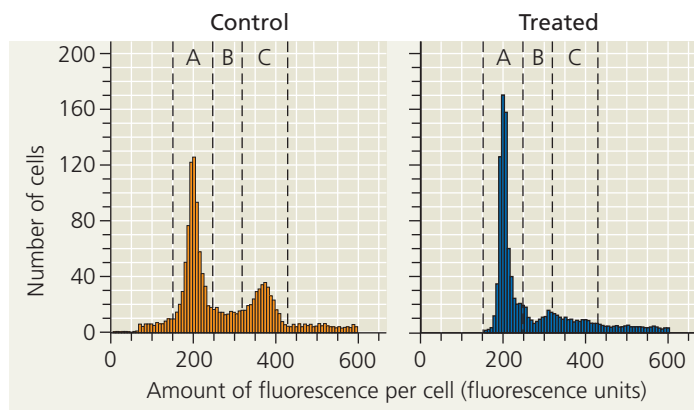
Interpreting Histograms

At What Phase Is the Cell Cycle Arrested by an Inhibitor?

Many medical treatments are aimed at stopping cancer cell proliferation by blocking the cell cycle of cancerous tumor cells. One potential treatment is a cell cycle inhibitor derived from human umbilical cord stem cells. In this exercise, you will compare two histograms to determine where in the cell cycle the inhibitor blocks the division of cancer cells.

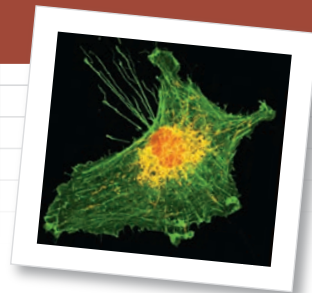
How the Experiment Was Done In the treated sample, human glioblastoma (brain cancer) cells were grown in tissue culture in the presence of the inhibitor, while a control sample of glioblastoma cells was grown in its absence. After 72 hours of growth, the two cell samples were harvested. To get a “snapshot” of the phase of the cell cycle each cell was in at that time, the samples were treated with a fluorescent chemical that binds to DNA and then run through a flow cytometer, an instrument that records the fluorescence level of each cell. Computer software then graphed the number of cells in each sample with a particular fluorescence level, as shown below.

Data from the Experiment



The data are plotted in a type of graph called a histogram (above), which groups the values for a numeric variable on the x-axis into intervals. A histogram allows you to see how all the experimental subjects (cells, in this case) are distributed along a continuous variable (amount of fluorescence). In these histograms, the bars

are so narrow that the data appear to follow a curve for which you can detect peaks and dips. Each narrow bar represents the number of cells observed to have a level of fluorescence in the range of that interval. This in turn indicates the relative amount of DNA in those cells. Overall, comparing the two histograms allows you to see how the DNA content of this cell population is altered by the treatment.



INTERPRET THE DATA

- Study the data in the histograms. (a) Which axis indirectly shows the relative amount of DNA per cell? Explain your answer. (b) In the control sample, compare the first peak in the histogram (in region A) to the second peak (in region C). Which peak shows the population of cells with the higher amount of DNA per cell? Explain. (For additional information about graphs, see the Scientific Skills Review in Appendix F.)
- (a) In the control sample histogram, identify the phase of the cell cycle (G_1 , S, or G_2) of the population of cells in each region delineated by vertical lines. Label the histogram with these phases and explain your answer. (b) Does the S phase population of cells show a distinct peak in the histogram? Why or why not?
- The histogram representing the treated sample shows the effect of growing the cancer cells alongside human umbilical cord stem cells that produce the potential inhibitor. (a) Label the histogram with the cell cycle phases. Which phase of the cell cycle has the greatest number of cells in the treated sample? Explain. (b) Compare the distribution of cells among G_1 , S, and G_2 phases in the control and treated samples. What does this tell you about the cells in the treated sample? (c) Based on what you learned in Concept 12.3, propose a mechanism by which the stem cell-derived inhibitor might arrest the cancer cell cycle at this stage. (More than one answer is possible.)



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Data from K. K. Velpula et al., Regulation of glioblastoma progression by cord blood stem cells is mediated by downregulation of cyclin D1, *PLoS ONE* 6(3):e18017 (2011).

Over the past several decades, researchers have produced a flood of valuable information about cell-signaling pathways and how their malfunction contributes to the development of cancer through effects on the cell cycle. Coupled with new molecular techniques, such as the ability to rapidly sequence the DNA of cells in a particular tumor, medical treatments for cancer are beginning to become more “personalized” to a particular patient’s tumor (see Make Connections Figure 18.27).

For example, the cells of roughly 20% of breast cancer tumors show abnormally high amounts of a cell-surface receptor tyrosine kinase called HER2, and many show an increase in the number of estrogen receptor (ER) molecules, intracellular receptors that can trigger cell division. Based on lab findings, a physician can prescribe chemotherapy with a molecule that blocks the function of the specific protein

(Herceptin for HER2 and tamoxifen for ERs). Treatment using these agents, when appropriate, has led to increased survival rates and fewer cancer recurrences.



Interview with Bruce Alberts: Cancer control and careers in science

CONCEPT CHECK 12.3

- In Figure 12.14, why do the nuclei resulting from experiment 2 contain different amounts of DNA?
- How does MPF allow a cell to pass the G_2 phase checkpoint and enter mitosis? (See Figure 12.16.)
- MAKE CONNECTIONS** Explain how receptor tyrosine kinases and intracellular receptors might function in triggering cell division. (Review Figures 9.8 and 9.9 and Concept 9.2.)

For suggested answers, see Appendix A.

12 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

- Unicellular organisms reproduce by **cell division**; multicellular organisms depend on cell division for their development from a fertilized egg and for growth and repair. Cell division is part of the **cell cycle**, an ordered sequence of events in the life of a cell.



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax

CONCEPT 12.1

Most cell division results in genetically identical daughter cells (pp. 285–287)

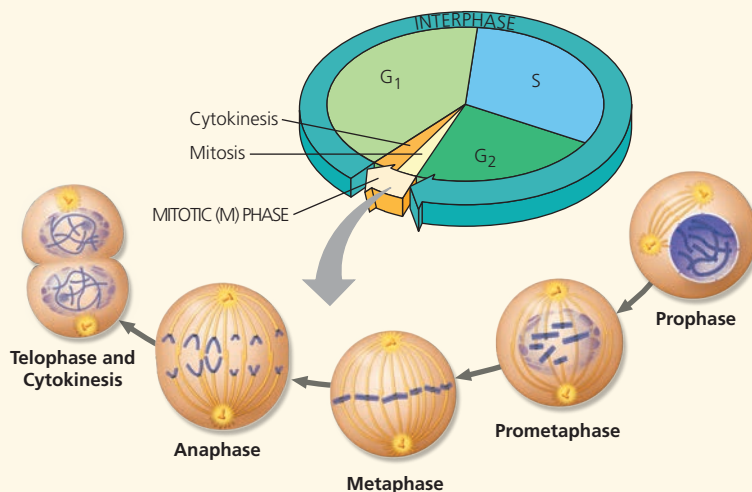
- The genetic material (DNA) of a cell—its **genome**—is partitioned among **chromosomes**. Each eukaryotic chromosome consists of one DNA molecule associated with many proteins. Together, the complex of DNA and associated proteins is called **chromatin**. The chromatin of a chromosome exists in different states of condensation at different times. In animals, **gametes** have one set of chromosomes and **somatic cells** have two sets.
- Cells replicate their genetic material before they divide, each daughter cell receiving a copy of the DNA. Prior to cell division, chromosomes are duplicated. Each one then consists of two identical **sister chromatids** joined along their lengths by sister chromatid cohesion and held most tightly together at a constricted region at the **centromeres**. When this cohesion is broken, the chromatids separate during cell division, becoming the chromosomes of the daughter cells. Eukaryotic cell division consists of **mitosis** (division of the nucleus) and **cytokinesis** (division of the cytoplasm).

? Differentiate between these terms: chromosome, chromatin, and chromatid.

CONCEPT 12.2

The mitotic phase alternates with interphase in the cell cycle (pp. 287–294)

- Between divisions, a cell is in **interphase**: the **G₁**, **S**, and **G₂** phases. The cell grows throughout interphase, with DNA being replicated only during the synthesis (S) phase. Mitosis and cytokinesis make up the **mitotic (M) phase** of the cell cycle.



- The **mitotic spindle**, made up of microtubules, controls chromosome movement during mitosis. In animal cells, it arises from the **centrosomes** and includes spindle microtubules and asters. Some spindle microtubules attach to the **kinetochores** of chromosomes and move the chromosomes to the **metaphase plate**. After sister chromatids separate, motor proteins move them along kinetochore microtubules toward opposite ends of the cell. The cell elongates when motor proteins push nonkinetochore microtubules from opposite poles away from each other.
- Mitosis is usually followed by cytokinesis. Animal cells carry out cytokinesis by **cleavage**, and plant cells form a **cell plate**.
- During **binary fission** in bacteria, the chromosome replicates and the daughter chromosomes actively move apart. Some of the proteins involved in bacterial binary fission are related to eukaryotic actin and tubulin.
- Since prokaryotes preceded eukaryotes by more than a billion years, it is likely that mitosis evolved from prokaryotic cell division. Certain unicellular eukaryotes exhibit mechanisms of cell division that may be similar to those of ancestors of existing eukaryotes. Such mechanisms might represent intermediate steps in the evolution of mitosis.

? In which of the three phases of interphase and the stages of mitosis do chromosomes exist as single DNA molecules?

CONCEPT 12.3

The eukaryotic cell cycle is regulated by a molecular control system (pp. 294–300)

- Signaling molecules present in the cytoplasm regulate progress through the cell cycle.
- The **cell cycle control system** is molecularly based. Cyclic changes in regulatory proteins work as a cell cycle clock. The key molecules are **cyclins** and **cyclin-dependent kinases (Cdks)**. The clock has specific **checkpoints** where the cell cycle stops until a go-ahead signal is received; important checkpoints occur in G₁, G₂, and M phases. Cell culture has enabled researchers to study the molecular details of cell division. Both internal signals and external signals control the cell cycle checkpoints via signal transduction pathways. Most cells exhibit **density-dependent inhibition** of cell division as well as **anchorage dependence**.
- Cancer cells elude normal cell cycle regulation and divide unchecked, forming tumors. **Malignant tumors** invade nearby tissues and can undergo **metastasis**, exporting cancer cells to other sites, where they may form secondary tumors. Recent cell cycle and cell signaling research, and new techniques for sequencing DNA, have led to improved cancer treatments.

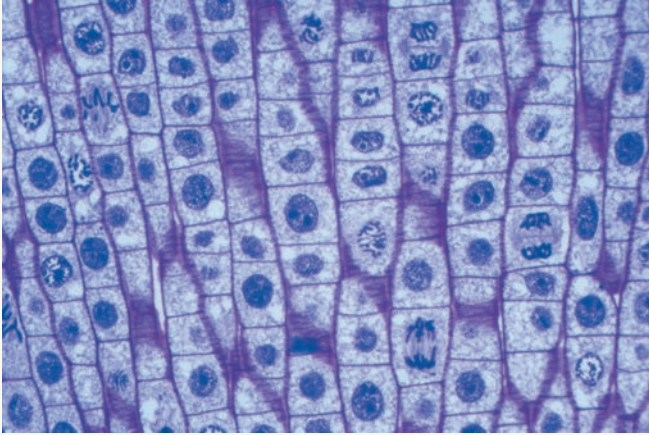
? Explain the significance of the G₁, G₂, and M checkpoints and the go-ahead signals involved in the cell cycle control system.

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–8 can be found in the Study Area in MasteringBiology.

9. **VISUAL SKILLS** The light micrograph shows dividing cells near the tip of an onion root. Identify a cell in each of the following stages: prophase, prometaphase, metaphase, anaphase, and telophase. Describe the major events occurring at each stage.

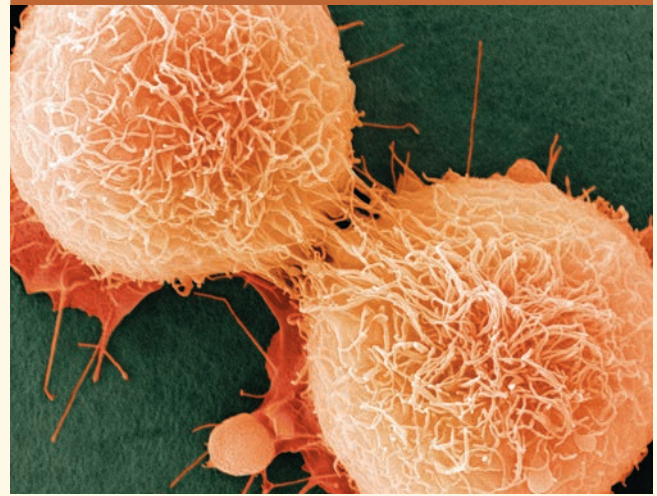


10. **DRAW IT** Draw one eukaryotic chromosome as it would appear during interphase, during each of the stages of mitosis, and during cytokinesis. Also draw and label the nuclear envelope and any microtubules attached to the chromosome(s).
11. **EVOLUTION CONNECTION** The result of mitosis is that the daughter cells end up with the same number of chromosomes that the parent cell had. Another potential way to maintain the number of chromosomes would be to carry out cell division first and then duplicate the chromosomes in each daughter cell. Assess whether this would be an equally good way of organizing the cell cycle. Explain why evolution has not led to this alternative.
12. **SCIENTIFIC INQUIRY** Although both ends of a microtubule can gain or lose subunits, one end (called the plus end) polymerizes and depolymerizes at a higher rate than the other end (the minus end). For spindle microtubules, the plus ends are in the center of the spindle, and the minus ends are at the poles. Motor proteins that move along microtubules

specialize in walking either toward the plus end or toward the minus end; the two types are called plus end-directed and minus end-directed motor proteins, respectively. Given what you know about chromosome movement and spindle changes during anaphase, predict which type of motor proteins would be present on (a) kinetochore microtubules and (b) nonkinetochore microtubules.

13. **WRITE ABOUT A THEME: INFORMATION** Unlike eukaryotes, most of the genes of bacteria are contained in a single, circular DNA molecule. In a short essay (100–150 words), explain how the circular bacterial genome is replicated and copies of the genome are distributed equally to two daughter cells.

14. **SYNTHESIZE YOUR KNOWLEDGE**



Shown here are two HeLa cancer cells that are just completing cytokinesis. Explain how the cell division of cancer cells like these is misregulated. Identify genetic and other changes that might have caused these cells to escape normal cell cycle regulation.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

UNIT 3

THE GENETIC BASIS OF LIFE

Born in Toronto, Canada, Shirley Tilghman majored in chemistry and biochemistry at Queen's University in Kingston, Ontario. She earned a Ph.D. in Biochemistry from Temple University in 1975. As a postdoctoral fellow at the National Institutes of Health, she helped clone the first mammalian gene (β -globin) and began to ask questions about the molecular basis for embryonic development. After her postdoc, she was a researcher at what is now the Fox Chase Cancer Center in Philadelphia. In 1986, she moved to Princeton University, where she made seminal discoveries about "imprinted" genes, genes that are expressed differently depending on the parent from which they are inherited. She was the founding director of the Lewis-Sigler Institute for Integrative Genomics. Dr. Tilghman was the president of Princeton from 2001 to 2013. She is a member of the National Academy of Sciences and serves as a trustee for multiple institutions.



An Interview with Shirley Tilghman

How did you get interested in science, and in biology in particular?

It began with loving math. My father claims that when I was five, I wanted to do mental math puzzles instead of having stories read to me! In high school this evolved into a strong interest in chemistry because I loved the logic of chemistry—the rules. But in college I realized I was unlikely to be a good chemist because it wasn't coming naturally to me. I went looking for something more compatible with the way my brain worked and happily stumbled upon the Meselson-Stahl experiment [see Figure 16.11], which I thought was the coolest thing I had ever read. I barely knew what DNA was, but I knew I wanted to learn about it! I wanted to be a molecular biologist.

When you set up your own molecular biology lab, how did you choose what to study?

I wanted to study how genes get turned on and off during early mammalian development, so I went looking through the literature for a messenger RNA that was expressed very early at very high levels and was regulated. I stumbled on the α -fetoprotein gene, the *Afp* gene. It's turned on early in fetal development and is one of the most abundant mRNAs, but is completely shut off after birth. We learned that it is the enhancers of this gene—the DNA sequences that transcription factors bind to—that make such high levels of gene expression possible during specific stages of development.

How did your work on regulation of *Afp* lead you to study the *H19* gene?

This was a classic case of serendipity. If you've seen the children's book *Little Bunny Follows His Nose*, that's how I think of the *H19* gene. When we were working on *Afp*, we wanted to find other genes regulated in the same way, and *H19* was the most dramatic example. The first thing you did then when you got a gene was to sequence it, and that's what my graduate student Vassilis Pachnis did. He brought the sequence to me, and I could see that there was no region in it that coded for a protein, so I asked him to sequence it again. That poor guy sequenced *H19* probably ten times! What finally convinced us that the gene's product was a "noncoding RNA"—an RNA that didn't code for a protein—was sequencing the same gene from multiple mammals. We saw that the sequence was very similar in all of them, but none of them had a sequence that coded for a protein. *H19* was the first gene for a noncoding RNA ever found!

What else did you find out about the *H19* gene?

When we were working on *H19*, I had a classic science experience. I went to a scientific meeting and met a researcher who had reported "imprinting" of the *Igf2* gene, which is near *H19*. Imprinting means that only the copy of the gene inherited from one particular parent is expressed. There were many parallels between *Igf2* and *H19*, so I ran back to the lab and convinced a postdoc named Marisa Bartolomei to do an experiment to determine whether *H19* is imprinted. We discovered that only the copy of the *H19* gene inherited from the mother is expressed—in other words, *H19* was imprinted also. We spent the next ten years working out the details. This was the first imprinted gene where the mechanism was unraveled. It turns out that imprinting involves adding methyl groups to the DNA that affect expression of the gene—the methyl groups are only added in the gamete from one parent, either the mother or the father. At least 100 genes are now known to be imprinted, and there are even more candidate genes to analyze.

"If you've seen the children's book *Little Bunny Follows His Nose*, that's how I think of the *H19* gene."

▼ Dr. Tilghman and graduate student Ekaterina Semenova examine a mouse tissue preparation.



Sexual Life Cycles and Meiosis

13



▲ **Figure 13.1** What accounts for family resemblance?

KEY CONCEPTS

- 13.1** Offspring acquire genes from parents by inheriting chromosomes
- 13.2** Fertilization and meiosis alternate in sexual life cycles
- 13.3** Meiosis reduces the number of chromosome sets from diploid to haploid
- 13.4** Genetic variation produced in sexual life cycles contributes to evolution

▼ A sperm fertilizing an egg.



Variations on a Theme

We all know that offspring resemble their parents more than they do unrelated individuals. If you examine the family members shown in **Figure 13.1**, you can pick out some similar features among them. The transmission of traits from one generation to the next is called inheritance, or **heredity** (from the Latin *heres*, heir). However, sons and daughters are not identical copies of either parent or of their siblings. Along with inherited similarity, there is also **variation**. What are the biological mechanisms leading to the “family resemblance” evident among the family members in the photo? A detailed answer to this question eluded biologists until the advance of genetics in the 20th century.

Genetics is the scientific study of heredity and inherited variation. In this unit, you’ll learn about genetics at multiple levels, from organisms to cells to molecules. We begin by examining how chromosomes pass from parents to offspring in sexually reproducing organisms. The processes of meiosis (a special type of cell division) and fertilization (the fusion of sperm and egg, as seen in the small photo) maintain a species’ chromosome count during the sexual life cycle. We will describe the cellular mechanics of meiosis and explain how this process differs from mitosis. Finally, we will consider how both meiosis and fertilization contribute to genetic variation, such as that seen in Figure 13.1.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

CONCEPT 13.1

Offspring acquire genes from parents by inheriting chromosomes

Family friends may tell you that you have your mother's nose or your father's eyes. Of course, parents do not, in any literal sense, give their children a nose, eyes, hair, or any other traits. What, then, *is* actually inherited?

Inheritance of Genes

Parents endow their offspring with coded information in the form of hereditary units called **genes**. The genes we inherit from our mothers and fathers are our genetic link to our parents, and they account for family resemblances such as shared eye color or freckles. Our genes program specific traits that emerge as we develop from fertilized eggs into adults.

The genetic program is written in the language of DNA, the polymer of four different nucleotides you learned about in Concepts 1.1 and 5.5. Inherited information is passed on in the form of each gene's specific sequence of DNA nucleotides, much as printed information is communicated in the form of meaningful sequences of letters. In both cases, the language is symbolic. Just as your brain translates the word *apple* into a mental image of the fruit, cells translate genes into freckles and other features. Most genes program cells to synthesize specific enzymes and other proteins, whose cumulative action produces an organism's inherited traits. The programming of these traits in the form of DNA is one of the unifying themes of biology.

The transmission of hereditary traits has its molecular basis in the replication of DNA, which produces copies of genes that can be passed from parents to offspring. In animals and plants, reproductive cells called **gametes** are the vehicles that transmit genes from one generation to the next. During fertilization, male and female gametes (sperm and eggs) unite, passing on genes of both parents to their offspring.

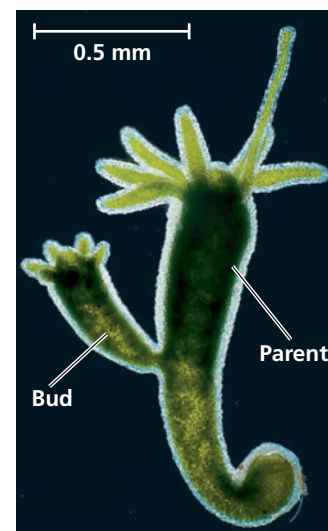
Except for small amounts of DNA in mitochondria and chloroplasts, the DNA of a eukaryotic cell is packaged into chromosomes within the nucleus. Every species has a characteristic number of chromosomes. For example, humans have 46 chromosomes in their **somatic cells**—all cells of the body except the gametes and their precursors. Each chromosome consists of a single long DNA molecule, elaborately coiled in association with various proteins. One chromosome includes several hundred to a few thousand genes, each of which is a precise sequence of nucleotides along the DNA molecule. A gene's specific location along the length of a chromosome is called the gene's **locus** (plural, *loci*; from the Latin, meaning "place"). Our genetic endowment (our genome) consists of the genes and other DNA that make up the chromosomes we inherited from our parents.

Comparison of Asexual and Sexual Reproduction

Only organisms that reproduce asexually have offspring that are exact genetic copies of themselves. In **asexual reproduction**, a single individual (like a yeast cell or an amoeba; see Figure 12.2a) is the sole parent and passes copies of all its genes to its offspring without the fusion of gametes. For example, single-celled eukaryotic organisms can reproduce asexually by mitotic cell division, in which DNA is copied and allocated equally to two daughter cells. The genomes of the offspring are virtually exact copies of the parent's genome. Some multicellular organisms are also capable of reproducing asexually (**Figure 13.2**). Because the cells of the offspring arise via mitosis in the parent, the offspring is usually genetically identical to its parent. An individual that reproduces asexually gives rise to a **clone**, a group of genetically identical individuals. Genetic differences occasionally arise in asexually reproducing organisms as a result of changes in the DNA called mutations, which we will discuss in Concept 17.5.

In **sexual reproduction**, two parents give rise to offspring that have unique combinations of genes inherited from the two parents. In contrast to a clone, offspring of sexual reproduction vary genetically from their siblings and both parents: They are variations on a common theme of family resemblance, not exact replicas. Genetic variation like that shown in Figure 13.1 is an important consequence of sexual reproduction. What mechanisms generate this genetic variation? The key is the behavior of chromosomes during the sexual life cycle.

✓ Figure 13.2 Asexual reproduction in two multicellular organisms. (a) This relatively simple animal, a hydra, reproduces by budding. The bud, a localized mass of mitotically dividing cells, develops into a small hydra, which detaches from the parent (LM). (b) All the trees in this circle of redwoods arose asexually from a single parent tree, whose stump is in the center of the circle.



(a) Hydra



(b) Redwoods



Video: Hydra Budding
Animation: Asexual Reproduction

CONCEPT CHECK 13.1

1. **MAKE CONNECTIONS** > Using what you know of gene expression in a cell, explain what causes the traits of parents (such as hair color) to show up in their offspring. (See Concept 5.5.)
2. How does an asexually reproducing eukaryotic organism produce offspring that are genetically identical to each other and to their parents?
3. **WHAT IF?** > A horticulturalist breeds orchids, trying to obtain a plant with a unique combination of desirable traits. After many years, she finally succeeds. To produce more plants like this one, should she crossbreed it with another plant or clone it? Why?

For suggested answers, see Appendix A.

CONCEPT 13.2

Fertilization and meiosis alternate in sexual life cycles

A **life cycle** is the generation-to-generation sequence of stages in the reproductive history of an organism, from conception to production of its own offspring. In this section, we use humans as an example to track the behavior of chromosomes through the sexual life cycle. We begin by considering the chromosome count in human somatic cells and gametes. We will then explore how the behavior of chromosomes relates to the human life cycle and other types of sexual life cycles.

Sets of Chromosomes in Human Cells

In humans, each somatic cell has 46 chromosomes. During mitosis, the chromosomes become condensed enough to be visible under a light microscope. At this point, they can be distinguished from one another by their size, the position of their centromeres, and the pattern of colored bands produced by certain chromatin-binding stains.

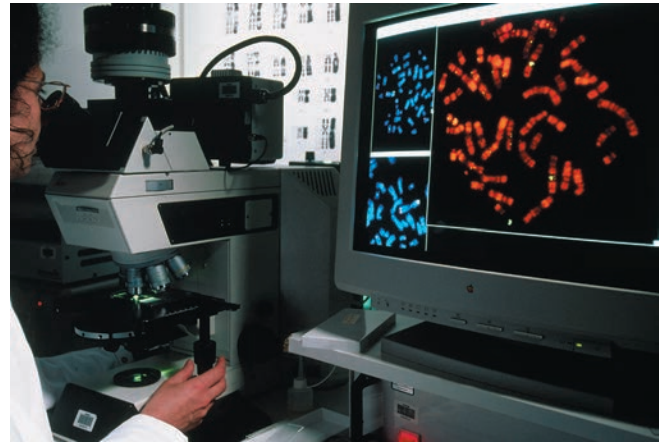
Careful examination of a micrograph of the 46 human chromosomes from a single cell in mitosis reveals that there are two chromosomes of each of 23 types. This becomes clear when images of the chromosomes are arranged in pairs, starting with the longest chromosomes. The resulting ordered display is called a **karyotype** (Figure 13.3). The two chromosomes of a pair have the same length, centromere position, and staining pattern: These are called **homologous chromosomes** (or **homologs**). Both chromosomes of each pair carry genes controlling the same inherited characters. For example, if a gene for eye color is situated at a particular locus on a certain chromosome, then its homologous chromosome (its homolog) will also have a version of the eye-color gene at the equivalent locus.

The two chromosomes referred to as X and Y are an important exception to the general pattern of homologous chromosomes in human somatic cells. Typically, human females have a homologous pair of X chromosomes (XX), while males have one X and one Y chromosome (XY; see Figure 13.3). Only small parts of the X and Y are homologous. Most of the

Figure 13.3

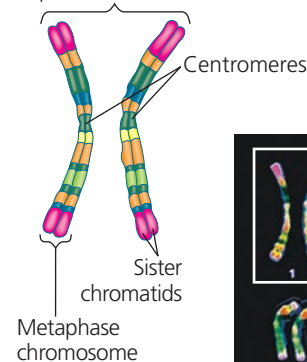
Research Method Preparing a Karyotype

Application A karyotype is a display of condensed chromosomes arranged in pairs. Karyotyping can be used to screen for defective chromosomes or abnormal numbers of chromosomes associated with certain congenital disorders, such as Down syndrome.



Technique Karyotypes are prepared from isolated somatic cells, which are treated with a drug to stimulate mitosis and then grown in culture for several days. Cells arrested when the chromosomes are most highly condensed—at metaphase—are stained and then viewed with a microscope equipped with a digital camera. An image of the chromosomes is displayed on a computer monitor, and digital software is used to arrange them in pairs according to their appearance.

Pair of homologous duplicated chromosomes



Results This karyotype shows the chromosomes from a human male (as seen by the presence of the XY chromosome pair), colored to emphasize their chromosome banding patterns. The size of the chromosome, position of the centromere, and pattern of stained bands help identify specific chromosomes. Although difficult to discern in the karyotype, each metaphase chromosome consists of two closely attached sister chromatids (see the diagram of the first pair of homologous duplicated chromosomes).

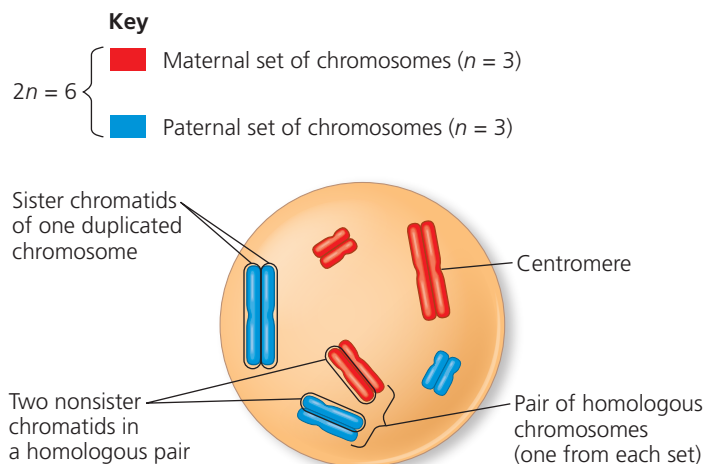
genes carried on the X chromosome do not have counterparts on the tiny Y, and the Y chromosome has genes lacking on the X. Due to their role in sex determination, the X and Y chromosomes are called **sex chromosomes**. The other chromosomes are called **autosomes**.

The occurrence of pairs of homologous chromosomes in each human somatic cell is a consequence of our sexual origins. We inherit one chromosome of a pair from each parent. Thus, the 46 chromosomes in our somatic cells are actually two sets of 23 chromosomes—a maternal set (from our mother) and a paternal set (from our father). The number of chromosomes in a single set is represented by n . Any cell with two chromosome sets is called a **diploid cell** and has a diploid number of chromosomes, abbreviated $2n$. For humans, the diploid number is 46 ($2n = 46$), the number of chromosomes in our somatic cells. In a cell in which DNA synthesis has occurred, all the chromosomes are duplicated, and therefore each consists of two identical sister chromatids, associated closely at the centromere and along the arms. (Even though the chromosomes are duplicated, we still say the cell is diploid, or $2n$. This is because it has only two sets of information regardless of the number of chromatids, which are merely copies of the information in one set.)

Figure 13.4 helps clarify the various terms that we use to describe duplicated chromosomes in a diploid cell.

Unlike somatic cells, gametes contain a single set of chromosomes. Such cells are called **haploid cells**, and each has a haploid number of chromosomes (n). For humans, the haploid number is 23 ($n = 23$). The set of 23 consists of the

▼ **Figure 13.4 Describing chromosomes.** A cell from an organism with a diploid number of 6 ($2n = 6$) is depicted here following chromosome duplication and condensation. Each of the six duplicated chromosomes consists of two sister chromatids associated closely along their lengths. Each homologous pair is composed of one chromosome from the maternal set (red) and one from the paternal set (blue). Each set is made up of three chromosomes in this example (long, medium, and short). Together, one maternal and one paternal chromatid in a pair of homologous chromosomes are called nonsister chromatids.



VISUAL SKILLS ► How many sets of chromosomes are present in this diagram? How many pairs of homologous chromosomes are present?

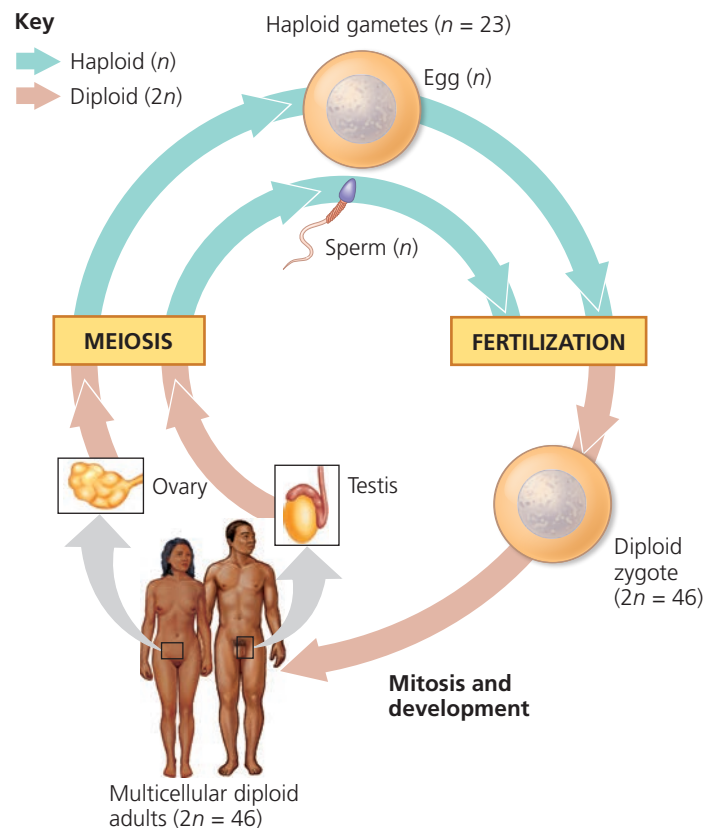
22 autosomes plus a single sex chromosome. An unfertilized egg contains an X chromosome; a sperm contains either an X or a Y chromosome.

Each sexually reproducing species has a characteristic diploid and haploid number. For example, the fruit fly *Drosophila melanogaster* has a diploid number ($2n$) of 8 and a haploid number (n) of 4, while for dogs, $2n$ is 78 and n is 39. The chromosome number generally does not correlate with the size or complexity of a species' genome; it simply reflects how many linear pieces of DNA make up the genome, which is a function of the evolutionary history of that species (see Concept 20.5). Now let's consider chromosome behavior during sexual life cycles. We'll use the human life cycle as an example.

Behavior of Chromosome Sets in the Human Life Cycle

The human life cycle begins when a haploid sperm from the father fuses with a haploid egg from the mother (**Figure 13.5**).

▼ **Figure 13.5 The human life cycle.** In each generation, the number of chromosome sets is halved during meiosis but doubles at fertilization. For humans, the number of chromosomes in a haploid cell is 23, consisting of one set ($n = 23$); the number of chromosomes in the diploid zygote and all somatic cells arising from it is 46, consisting of two sets ($2n = 46$).



This figure introduces a color code that will be used for other life cycles later in this book. The aqua arrows identify haploid stages of a life cycle, and the tan arrows identify diploid stages.

This union of gametes, culminating in fusion of their nuclei, is called **fertilization**. The resulting fertilized egg, or **zygote**, is diploid because it contains two haploid sets of chromosomes bearing genes representing the maternal and paternal family lines. As a human develops into a sexually mature adult, mitosis of the zygote and its descendant cells generates all the somatic cells of the body. Both chromosome sets in the zygote and all the genes they carry are passed with precision to the somatic cells.

The only cells of the human body not produced by mitosis are the gametes, which develop from specialized cells called *germ cells* in the gonads—ovaries in females and testes in males (see Figure 13.5). Imagine what would happen if human gametes were made by mitosis: They would be diploid like the somatic cells. At the next round of fertilization, when two gametes fused, the normal chromosome number of 46 would double to 92, and each subsequent generation would double the number of chromosomes yet again. This does not happen, however, because in sexually reproducing organisms, gamete formation involves a type of cell division called **meiosis**. This type of cell division reduces the number of sets of chromosomes from two to one in the gametes, counterbalancing the doubling that occurs at fertilization. As a result of meiosis, each human sperm and egg is haploid ($n = 23$). Fertilization restores the diploid condition by combining two sets of chromosomes, and the human life cycle is repeated, generation after generation (see Figure 13.5).

In general, the steps of the human life cycle are typical of many sexually reproducing animals. Indeed, the processes of fertilization and meiosis are also the hallmarks of sexual reproduction in plants, fungi, and protists just as in animals.

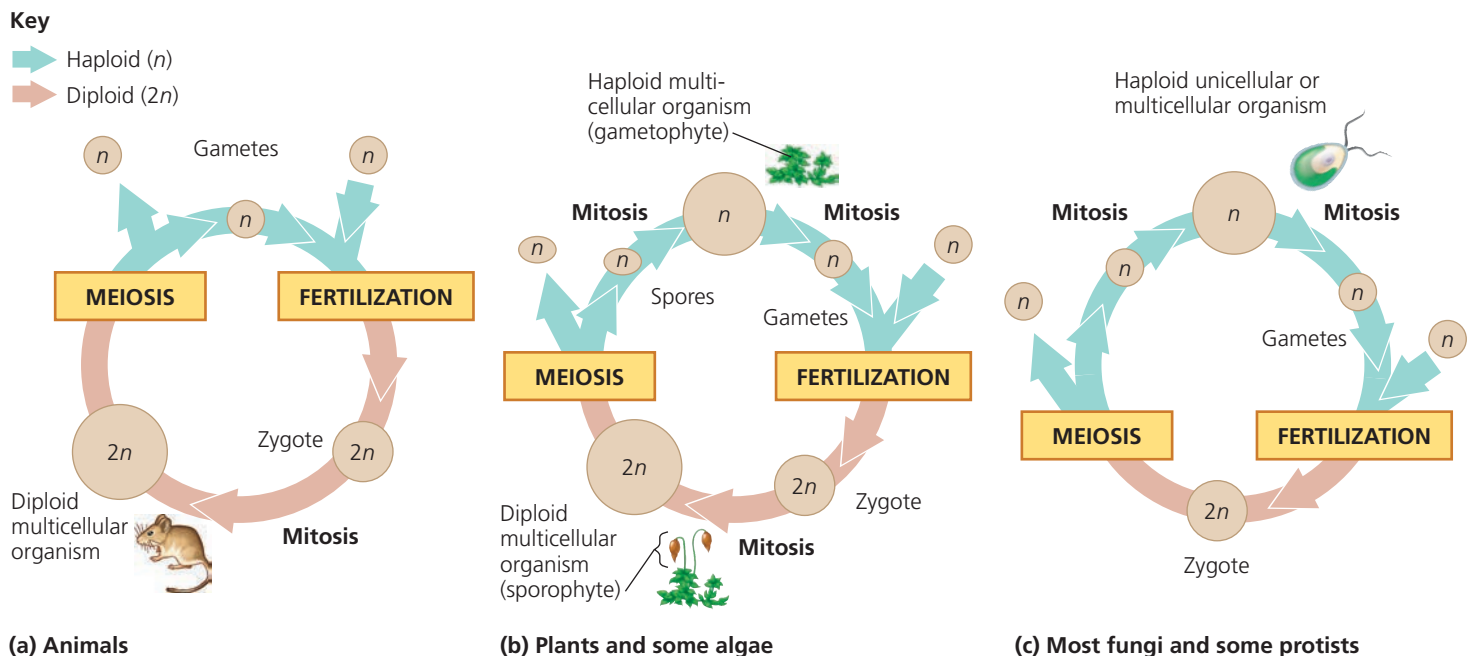
Fertilization and meiosis alternate in sexual life cycles, maintaining a constant number of chromosomes in each species from one generation to the next.

The Variety of Sexual Life Cycles

Although the alternation of meiosis and fertilization is common to all organisms that reproduce sexually, the timing of these two events in the life cycle varies, depending on the species. These variations can be grouped into three main types of life cycles. In the type that occurs in humans and most other animals, gametes are the only haploid cells (**Figure 13.6a**). Meiosis occurs in germ cells during the production of gametes, which undergo no further cell division prior to fertilization. After fertilization, the diploid zygote divides by mitosis, producing a multicellular organism that is diploid.

Plants and some species of algae exhibit a second type of life cycle called **alternation of generations** (**Figure 13.6b**). This type includes both diploid and haploid stages that are multicellular. The multicellular diploid stage is called the *sporophyte*. Meiosis in the sporophyte produces haploid cells called *spores*. Unlike a gamete, a haploid spore doesn't fuse with another cell but divides mitotically, generating a multicellular haploid stage called the *gametophyte*. Cells of the gametophyte give rise to gametes by mitosis. Fusion of two haploid gametes at fertilization results in a diploid zygote, which develops into the next sporophyte generation. Therefore, in this type of life cycle, the sporophyte generation produces a gametophyte as its offspring, and the gametophyte generation produces the next sporophyte generation (see Figure 13.6b). The term *alternation of generations* fits well as a name for this type of life cycle.

▼ **Figure 13.6 Three types of sexual life cycles.** The common feature of all three cycles is the alternation of meiosis and fertilization, key events that contribute to genetic variation among offspring. The cycles differ in the timing of these two key events. (Small circles are cells; large circles are organisms.)



VISUAL SKILLS ► For each type of life cycle, indicate whether haploid cells undergo mitosis, and if they do, describe the cells that are formed.

A third type of life cycle occurs in most fungi and some protists, including some algae (**Figure 13.6c**). After gametes fuse and form a diploid zygote, meiosis occurs without a multicellular diploid offspring developing. Meiosis produces not gametes but haploid cells that then divide by mitosis and give rise to either unicellular descendants or a haploid multicellular adult organism. Subsequently, the haploid organism carries out further mitoses, producing the cells that develop into gametes. The only diploid stage found in these species is the single-celled zygote.

Note that *either* haploid or diploid cells can divide by mitosis, depending on the type of life cycle. Only diploid cells, however, can undergo meiosis because haploid cells have only a single set of chromosomes that cannot be further reduced. Though the three types of sexual life cycles differ in the timing of meiosis and fertilization, they share a fundamental result: genetic variation among offspring.

CONCEPT CHECK 13.2

- 1. MAKE CONNECTIONS** > In Figure 13.4, how many DNA molecules (double helices) are present (see Figure 12.5)? What is the haploid number of this cell? Is a set of chromosomes haploid or diploid?
- 2. VISUAL SKILLS** > In the karyotype shown in Figure 13.3, how many pairs of chromosomes are present? How many sets?
- 3. WHAT IF?** > A certain eukaryote lives as a unicellular organism, but during environmental stress, it produces gametes. The gametes fuse, and the resulting zygote undergoes meiosis, generating new single cells. What type of organism could this be?

For suggested answers, see Appendix A.

CONCEPT 13.3

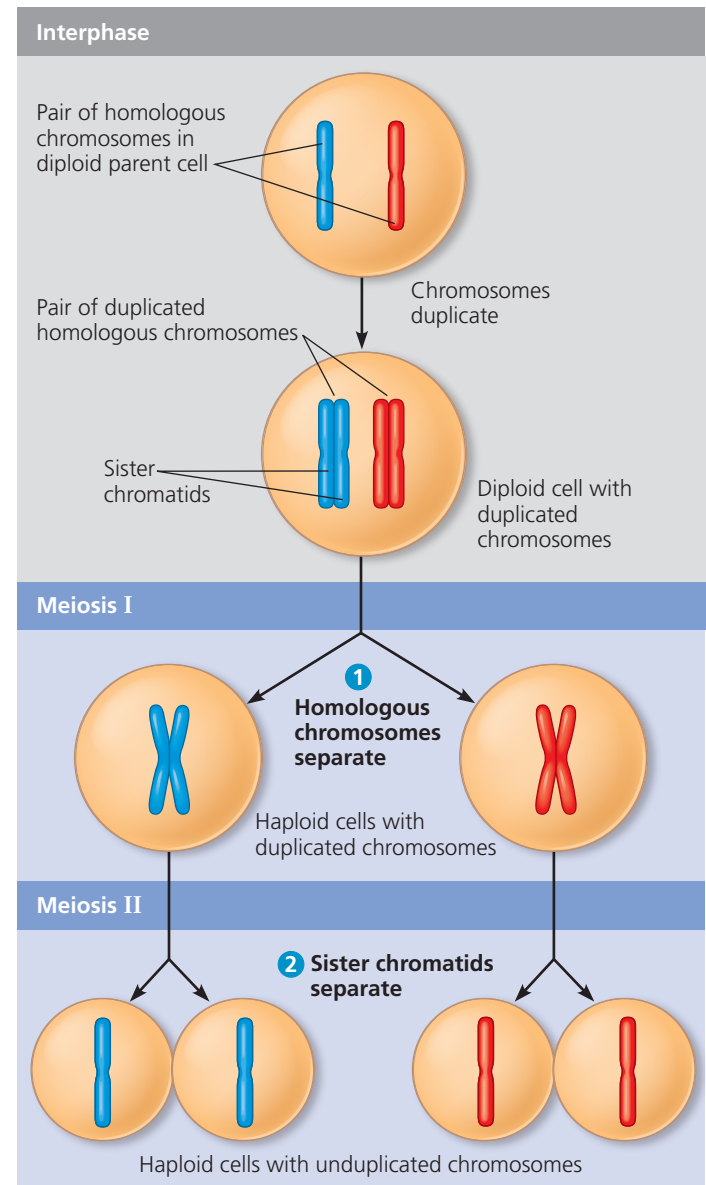
Meiosis reduces the number of chromosome sets from diploid to haploid

Several steps of meiosis closely resemble corresponding steps in mitosis. Meiosis, like mitosis, is preceded by the duplication of chromosomes. However, this single duplication is followed by not one but two consecutive cell divisions, called **meiosis I** and **meiosis II**. These two divisions result in four daughter cells (rather than the two daughter cells of mitosis), each with only half as many chromosomes as the parent cell—one set, rather than two.

The Stages of Meiosis

The overview of meiosis in **Figure 13.7** shows, for a single pair of homologous chromosomes in a diploid cell, that both members of the pair are duplicated and the copies sorted into four haploid daughter cells. Recall that sister chromatids are two copies of *one* chromosome, closely associated all along their lengths; this association is called *sister chromatid cohesion*. Together, the sister chromatids make up one duplicated

✓ Figure 13.7 Overview of meiosis: how meiosis reduces chromosome number. After the chromosomes duplicate in interphase, the diploid cell divides *twice*, yielding four haploid daughter cells. This overview tracks just one pair of homologous chromosomes, which for the sake of simplicity are drawn in the condensed state throughout.



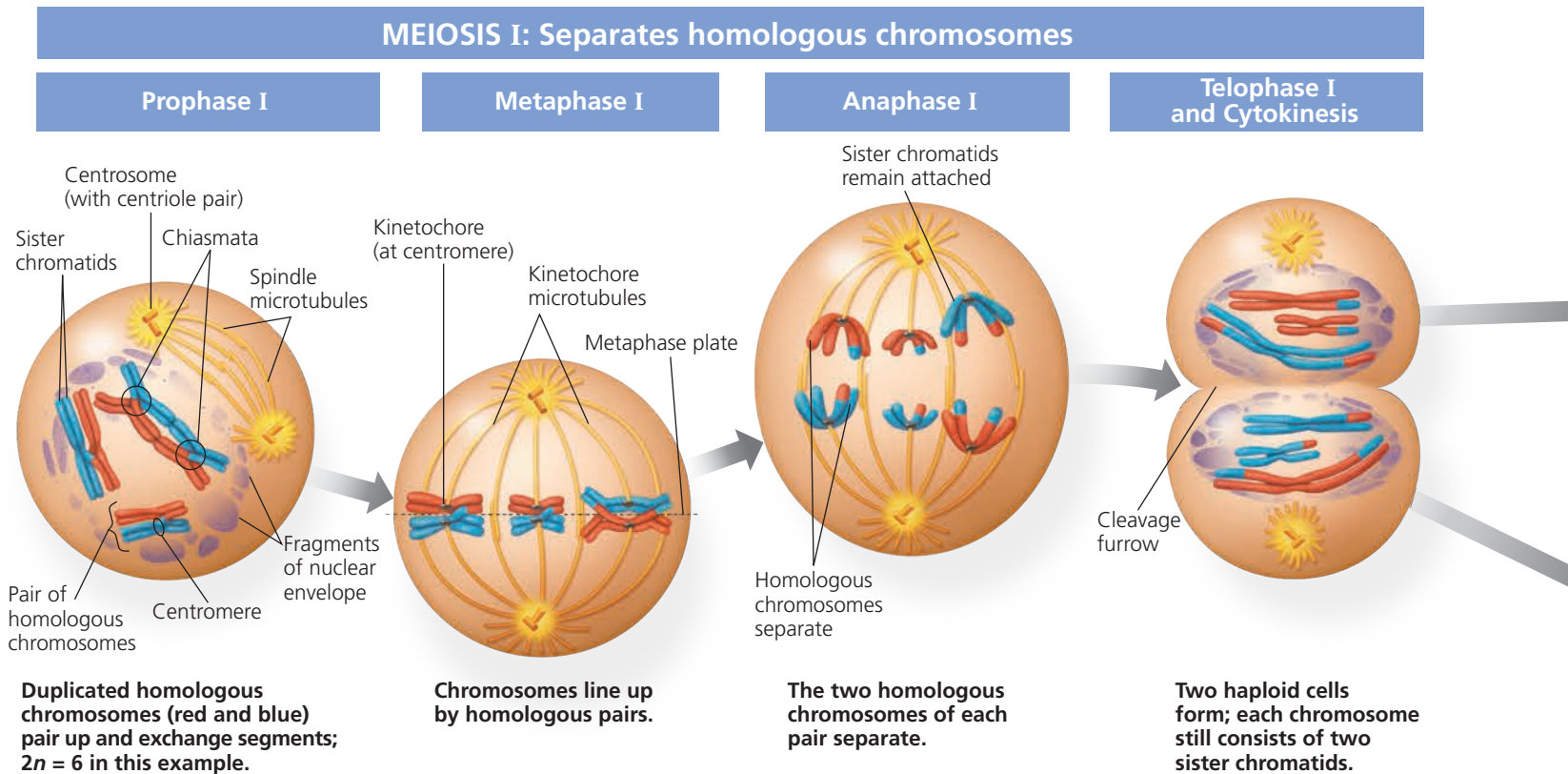
DRAW IT > Redraw the cells in this figure using a simple double helix to represent each DNA molecule.

Animation: Overview of Meiosis

chromosome (see Figure 13.4). In contrast, the two chromosomes of a homologous pair are individual chromosomes that were inherited from each parent. Homologs appear alike in the microscope, but they may have different versions of genes at corresponding loci; each version is called an *allele* of that gene (see Figure 14.4). Homologs are not associated with each other in any obvious way except during meiosis.

Figure 13.8 describes in detail the stages of the two divisions of meiosis for an animal cell whose diploid number is 6. Study this figure thoroughly before going on.

▼ Figure 13.8 Exploring Meiosis in an Animal Cell



Prophase I

- Centrosome movement, spindle formation, and nuclear envelope breakdown occur as in mitosis. Chromosomes condense progressively throughout prophase I.
- During early prophase I, before the stage shown above, each chromosome pairs with its homolog, aligned gene by gene, and **crossing over** occurs: The DNA molecules of nonsister chromatids are broken (by proteins) and are rejoined to each other.
- At the stage shown above, each homologous pair has one or more X-shaped regions called **chiasmata** (singular, *chiasma*), where crossovers have occurred.
- Later in prophase I, microtubules from one pole or the other attach to the kinetochores, one at the centromere of each homolog. (The two kinetochores on the sister chromatids of a homolog are linked together by proteins and act as a single kinetochore.) Microtubules move the homologous pairs toward the metaphase plate (see the metaphase I diagram).

Metaphase I

- Pairs of homologous chromosomes are now arranged at the metaphase plate, with one chromosome of each pair facing each pole.
- Both chromatids of one homolog are attached to kinetochore microtubules from one pole; the chromatids of the other homolog are attached to microtubules from the opposite pole.

Anaphase I

- Breakdown of proteins that are responsible for sister chromatid cohesion along chromatid arms allows homologs to separate.
- The homologs move toward opposite poles, guided by the spindle apparatus.
- Sister chromatid cohesion persists at the centromere, causing chromatids to move as a unit toward the same pole.

Telophase I and Cytokinesis

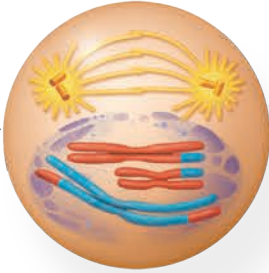
- When telophase I begins, each half of the cell has a complete haploid set of duplicated chromosomes. Each chromosome is composed of two sister chromatids; one or both chromatids include regions of nonsister chromatid DNA.
- Cytokinesis (division of the cytoplasm) usually occurs simultaneously with telophase I, forming two haploid daughter cells.
- In animal cells like these, a cleavage furrow forms. (In plant cells, a cell plate forms.)
- In some species, chromosomes decondense and nuclear envelopes form.
- No chromosome duplication occurs between meiosis I and meiosis II.



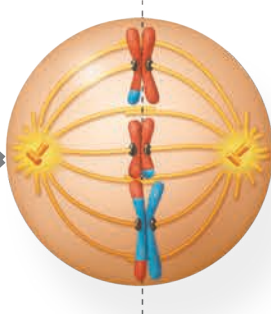
Video: Meiosis I in Sperm Formation

MEIOSIS II: Separates sister chromatids

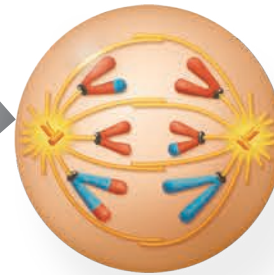
Prophase II



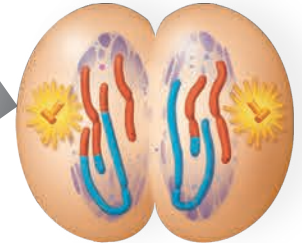
Metaphase II



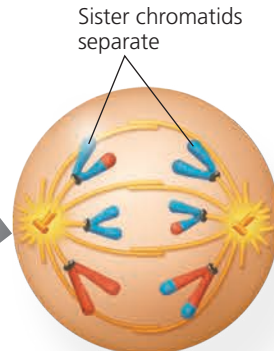
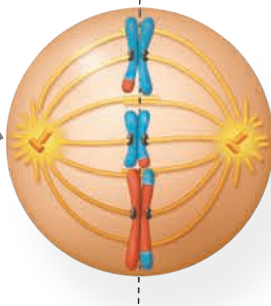
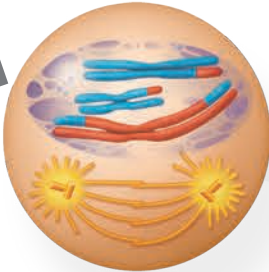
Anaphase II



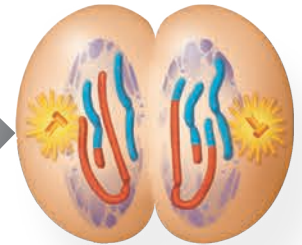
Telophase II and Cytokinesis



During another round of cell division, the sister chromatids finally separate; four haploid daughter cells result, containing unduplicated chromosomes.



Haploid daughter cells forming



Prophase II

- A spindle apparatus forms.
- In late prophase II (not shown here), chromosomes, each still composed of two chromatids associated at the centromere, are moved by microtubules toward the metaphase II plate.

Metaphase II

- The chromosomes are positioned at the metaphase plate as in mitosis.
- Because of crossing over in meiosis I, the two sister chromatids of each chromosome are not genetically identical.
- The kinetochores of sister chromatids are attached to microtubules extending from opposite poles.

Anaphase II

- Breakdown of proteins holding the sister chromatids together at the centromere allows the chromatids to separate. The chromatids move toward opposite poles as individual chromosomes.

Telophase II and Cytokinesis

- Nuclei form, the chromosomes begin decondensing, and cytokinesis occurs.
- The meiotic division of one parent cell produces four daughter cells, each with a haploid set of (unduplicated) chromosomes.
- The four daughter cells are genetically distinct from one another and from the parent cell.

MAKE CONNECTIONS > Look at Figure 12.7 and imagine the two daughter cells undergoing another round of mitosis, yielding four cells. Compare the number of chromosomes in each of those four cells, after mitosis, with the number in each cell in Figure 13.8, after meiosis. What is it about the process of meiosis that accounts for this difference, even though meiosis also includes two cell divisions?



BioFlix® Animation: Meiosis
Animation: Meiosis

Crossing Over and Synapsis During Prophase I

Prophase I of meiosis is a very busy time. The prophase I cell shown in Figure 13.8 is at a point fairly late in prophase I, when pairing of homologous chromosomes, crossing over, and chromosome condensation have already taken place. The sequence of events leading up to that point is shown in more detail in **Figure 13.9**.

After interphase, the chromosomes have been duplicated and the sister chromatids are held together by proteins called *cohesins*. **1** Early in prophase I, the two members of a homologous pair associate loosely along their length. Each gene on one homolog is aligned precisely with the corresponding allele of that gene on the other homolog. The DNA of two nonsister chromatids—one maternal and one paternal—is broken by specific proteins at precisely matching points. **2** Next, the formation of a zipper-like structure called the **synaptonemal complex** holds one homolog tightly to the other. **3** During this association, called **synapsis**, the DNA breaks are closed up so that each broken end is joined to the corresponding segment of the *nonsister* chromatid. Thus, a paternal chromatid is joined to a piece of maternal chromatid beyond the crossover point, and vice versa.

4 These points of crossing over become visible as **chiasmata** (singular, *chiasma*) after the synaptonemal complex disassembles and the homologs move slightly apart from each other. The homologs remain attached because sister chromatids are still held together by sister chromatid cohesion, even though some of the DNA may no longer be attached to its original chromosome. At least one crossover per chromosome must occur in order for the homologous pair to stay together as it moves to the metaphase I plate, for reasons that will be explained shortly.

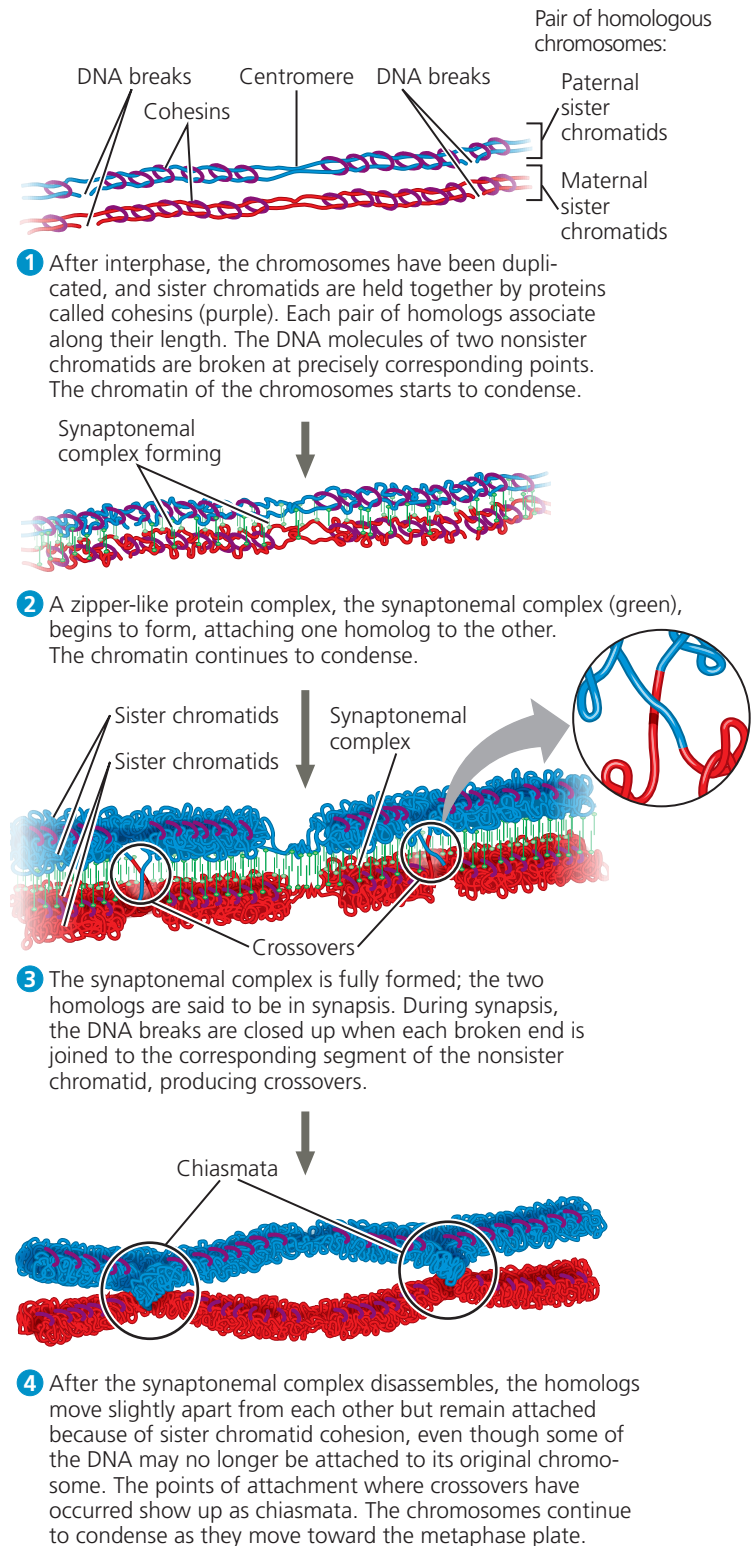
A Comparison of Mitosis and Meiosis

Figure 13.10 summarizes the key differences between meiosis and mitosis in diploid cells. Basically, meiosis reduces the number of chromosome sets from two (diploid) to one (haploid), whereas mitosis conserves the number of chromosome sets. Therefore, meiosis produces cells that differ genetically from their parent cell and from each other, whereas mitosis produces daughter cells that are genetically identical to their parent cell and to each other.

Three events unique to meiosis occur during meiosis I:

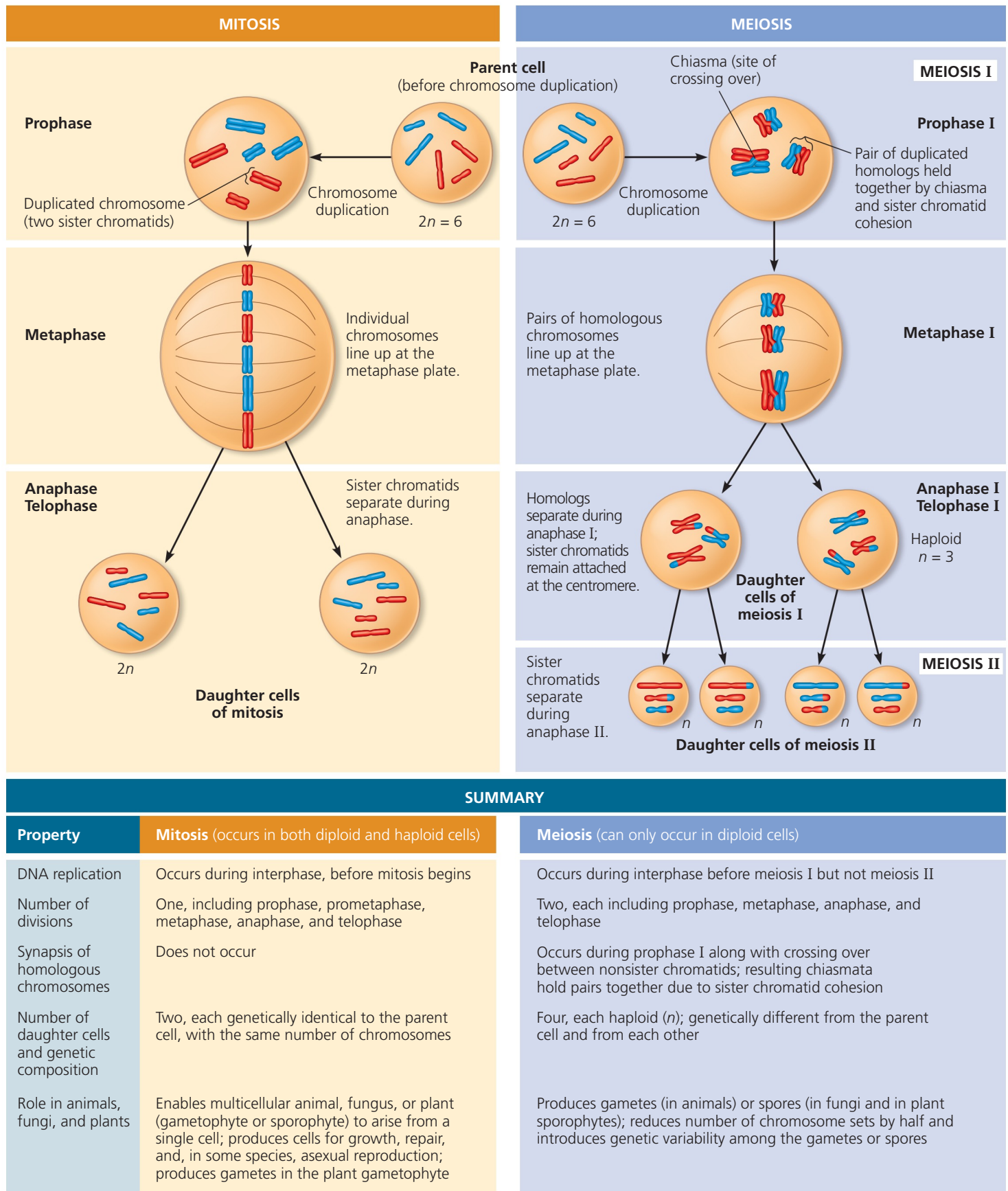
- 1. Synapsis and crossing over.** During prophase I, duplicated homologs pair up and crossing over occurs, as described previously and in Figure 13.9. Synapsis and crossing over do not occur during prophase of mitosis.
- 2. Alignment of homologous pairs at the metaphase plate.** At metaphase I of meiosis, pairs of homologs are positioned at the metaphase plate, rather than individual chromosomes, as in metaphase of mitosis.

▼ **Figure 13.9** Crossing over and synapsis in prophase I: a closer look.



- 3. Separation of homologs.** At anaphase I of meiosis, the duplicated chromosomes of each homologous pair move toward opposite poles, but the sister chromatids of each duplicated chromosome remain attached. In anaphase of mitosis, by contrast, sister chromatids separate.

▼ **Figure 13.10** A comparison of mitosis and meiosis.



DRAW IT ► Could any other combinations of chromosomes be generated during meiosis II from the specific cells shown in telophase I? Explain. (Hint: Draw the cells as they would appear in metaphase II.)

Sister chromatids stay together due to sister chromatid cohesion, mediated by cohesin proteins. In mitosis, this attachment lasts until the end of metaphase, when enzymes cleave the cohesins, freeing the sister chromatids to move to opposite poles of the cell. In meiosis, sister chromatid cohesion is released in two steps, one at the start of anaphase I and one at anaphase II. In metaphase I, the two homologs of each pair are held together because there is still cohesion between sister chromatid arms in regions beyond points of crossing over, where stretches of sister chromatids now belong to different chromosomes. The combination of crossing over and sister chromatid cohesion along the arms results in the formation of a chiasma. Chiasmata hold homologs together as the spindle forms for the first meiotic division. At the onset of anaphase I, the release of cohesion

along sister chromatid *arms* allows homologs to separate. At anaphase II, the release of sister chromatid cohesion at the *centromeres* allows the sister chromatids to separate. Thus, sister chromatid cohesion and crossing over, acting together, play an essential role in the lining up of chromosomes by homologous pairs at metaphase I.

Meiosis I reduces the number of chromosome sets: from two (diploid) to one (haploid). During the second meiotic division, sister chromatids separate, producing haploid daughter cells. The mechanisms for separating sister chromatids in meiosis II and mitosis are virtually identical. The molecular basis of chromosome behavior during meiosis continues to be a focus of intense research. In the **Scientific Skills Exercise**, you can work with data tracking the amount of DNA in cells as they progress through meiosis.

SCIENTIFIC SKILLS EXERCISE

Making a Line Graph and Converting Between Units of Data

How Does DNA Content Change as Budding Yeast Cells Proceed Through Meiosis? When nutrients are low, cells of the budding yeast (*Saccharomyces cerevisiae*) exit the mitotic cell cycle and enter meiosis. In this exercise, you will track the DNA content of a population of yeast cells as they progress through meiosis.

How the Experiment Was Done Researchers grew a culture of yeast cells in a nutrient-rich medium and then transferred the cells to a nutrient-poor medium to induce meiosis. At different times after induction, the DNA content per cell was measured in a sample of the cells, and the average DNA content per cell was recorded in femtograms (fg; $1 \text{ fg} = 1 \times 10^{-15} \text{ gram}$).

Data from the Experiment

Time After Induction (hours)	Average Amount of DNA per Cell (fg)
0.0	24.0
1.0	24.0
2.0	40.0
3.0	47.0
4.0	47.5
5.0	48.0
6.0	48.0
7.0	47.5
7.5	25.0
8.0	24.0
9.0	23.5
9.5	14.0
10.0	13.0
11.0	12.5
12.0	12.0
13.0	12.5
14.0	12.0

► Budding yeast cells



INTERPRET THE DATA

- First, set up your graph. (a) Place the labels for the independent and dependent variables on the appropriate axes, followed by units of measurement in parentheses. Explain your choices. (b) Add tick marks and values for each axis. Explain your choices. (For additional information about graphs, see the Scientific Skills Review in Appendix F.)
- Because the variable on the x-axis varies continuously, it makes sense to plot the data on a line graph. (a) Plot each data point from the table onto the graph. (b) Connect the data points with line segments.
- Most of the yeast cells in the culture were in G_1 of the cell cycle before being moved to the nutrient-poor medium. (a) How many femtograms of DNA are there in each yeast cell in G_1 ? Estimate this value from the data in your graph. (b) How many femtograms of DNA should be present in each cell in G_2 ? (See Concept 12.2 and Figure 12.6.) At the end of meiosis I (MI)? At the end of meiosis II (MII)? (See Figure 13.7.) (c) Using these values as a guideline, distinguish the different phases by inserting vertical dashed lines in the graph between phases and label each phase (G_1 , S, G_2 , MI, MII). You can figure out where to put the dividing lines based on what you know about the DNA content of each phase (see Figure 13.7). (d) Think carefully about the point where the line at the highest value begins to slope downward. What specific point of meiosis does this "corner" represent? What stage(s) correspond to the downward sloping line?
- Given the fact that $1 \text{ fg of DNA} = 9.78 \times 10^5 \text{ base pairs}$ (on average), you can convert the amount of DNA per cell to the length of DNA in numbers of base pairs. (a) Calculate the number of base pairs of DNA in the haploid yeast genome. Express your answer in millions of base pairs (Mb), a standard unit for expressing genome size. Show your work. (b) How many base pairs per minute were synthesized during the S phase of these yeast cells?



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Further Reading G. Simchen, Commitment to meiosis: what determines the mode of division in budding yeast? *BioEssays* 31:169–177 (2009).

CONCEPT CHECK 13.3

1. **MAKE CONNECTIONS** > Compare the chromosomes in a cell at metaphase of mitosis with those in a cell at metaphase II. (See Figures 12.7 and 13.8.)
2. **WHAT IF?** > After the synaptonemal complex disappears, how would any pair of homologous chromosomes be associated if crossing over did not occur? What effect might this have on gamete formation?

For suggested answers, see Appendix A.

CONCEPT 13.4

Genetic variation produced in sexual life cycles contributes to evolution

How do we account for the genetic variation of the family members in Figure 13.1? As you will learn more about in later chapters, mutations are the original source of genetic diversity. These changes in an organism's DNA create the different versions of genes, known as alleles. Once these differences arise, reshuffling of the alleles during sexual reproduction produces the variation that results in each member of a sexually reproducing population having a unique combination of traits.

Origins of Genetic Variation Among Offspring

In species that reproduce sexually, the behavior of chromosomes during meiosis and fertilization is responsible for most of the variation that arises in each generation. Three mechanisms contribute to the genetic variation arising from sexual reproduction: independent assortment of chromosomes, crossing over, and random fertilization.

Independent Assortment of Chromosomes

One aspect of sexual reproduction that generates genetic variation is the random orientation of pairs of homologous chromosomes at metaphase of meiosis I. At metaphase I, the homologous pairs, each consisting of one maternal and one paternal chromosome, are situated at the metaphase plate. (Note that the terms *maternal* and *paternal* refer, respectively, to whether the chromosome in question was contributed by the mother or the father of the individual whose cells are undergoing meiosis.) Each pair may orient with either its maternal or paternal homolog closer to a given pole—its orientation is as random as the flip of a coin. Thus, there is a 50% chance that a particular daughter cell of meiosis I will get the maternal chromosome of a certain homologous pair and a 50% chance that it will get the paternal chromosome.

Because each pair of homologous chromosomes is positioned independently

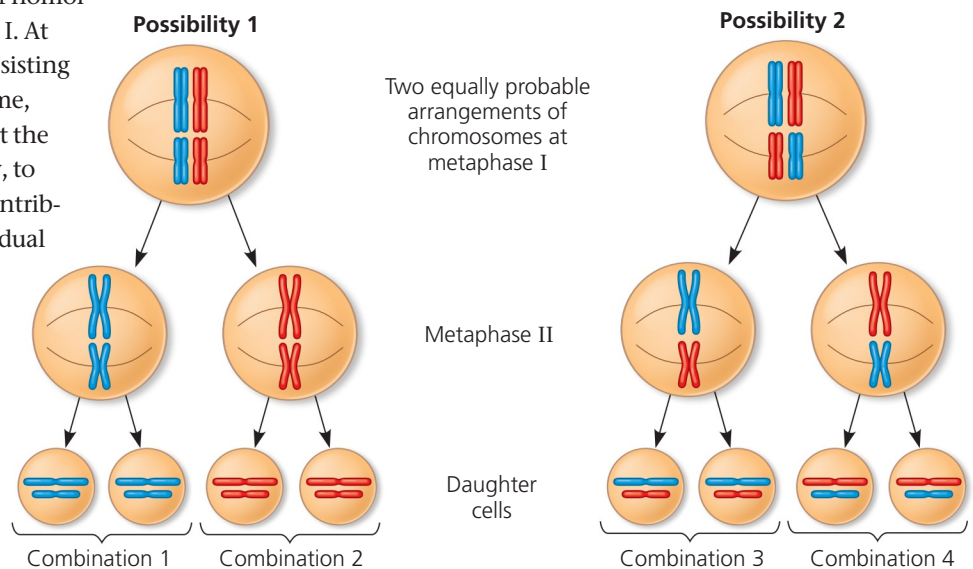
of the other pairs at metaphase I, the first meiotic division results in each pair sorting its maternal and paternal homologs into daughter cells independently of every other pair. This is called *independent assortment*. Each daughter cell represents one outcome of all possible combinations of maternal and paternal chromosomes. As shown in Figure 13.11, the number of combinations possible for daughter cells formed by meiosis of a diploid cell with two pairs of homologous chromosomes ($n = 2$) is four: two possible arrangements for the first pair times two possible arrangements for the second pair. Note that only two of the four combinations of daughter cells shown in the figure would result from meiosis of a *single* diploid cell, because a single parent cell would have one or the other possible chromosomal arrangement at metaphase I, but not both. However, the population of daughter cells resulting from meiosis of a large number of diploid cells contains all four types in approximately equal numbers. In the case of $n = 3$, eight combinations ($2 \times 2 \times 2 = 2^3$) of chromosomes are possible for daughter cells. More generally, the number of possible combinations when chromosomes sort independently during meiosis is 2^n , where n is the haploid number of the organism.

In the case of humans ($n = 23$), the number of possible combinations of maternal and paternal chromosomes in the resulting gametes is 2^{23} , or about 8.4 million. Each gamete that you produce in your lifetime contains one of roughly 8.4 million possible combinations of chromosomes. This is an underestimate, because it doesn't take into account crossing over, which we'll consider next.

Crossing Over

As a consequence of the independent assortment of chromosomes during meiosis, each of us produces a collection

✓ **Figure 13.11** The independent assortment of homologous chromosomes in meiosis.



Animation: Genetic Variation from Independent Assortment of Chromosomes

of gametes differing greatly in their combinations of the chromosomes we inherited from our two parents. Figure 13.11 suggests that each chromosome in a gamete is exclusively maternal or paternal in origin. In fact, this is *not* the case, because crossing over produces **recombinant chromosomes**, individual chromosomes that carry genes (DNA) from two different parents (**Figure 13.12**). In meiosis in humans, an average of one to three crossover events occurs per chromosome pair, depending on the size of the chromosomes and the position of their centromeres.

As you learned in Figure 13.9, crossing over produces chromosomes with new combinations of maternal and paternal alleles. At metaphase II, chromosomes that contain one or more recombinant chromatids can be oriented in two alternative, nonequivalent ways with respect to other chromosomes because their sister chromatids are no longer identical (see Figure 13.12). The different possible arrangements of non-identical sister chromatids during meiosis II further increase the number of genetic types of daughter cells that can result from meiosis.

You'll learn more about crossing over in Chapter 15. The important point for now is that crossing over, by combining DNA inherited from two parents into a single chromosome, is an important source of genetic variation in sexual life cycles.

Random Fertilization

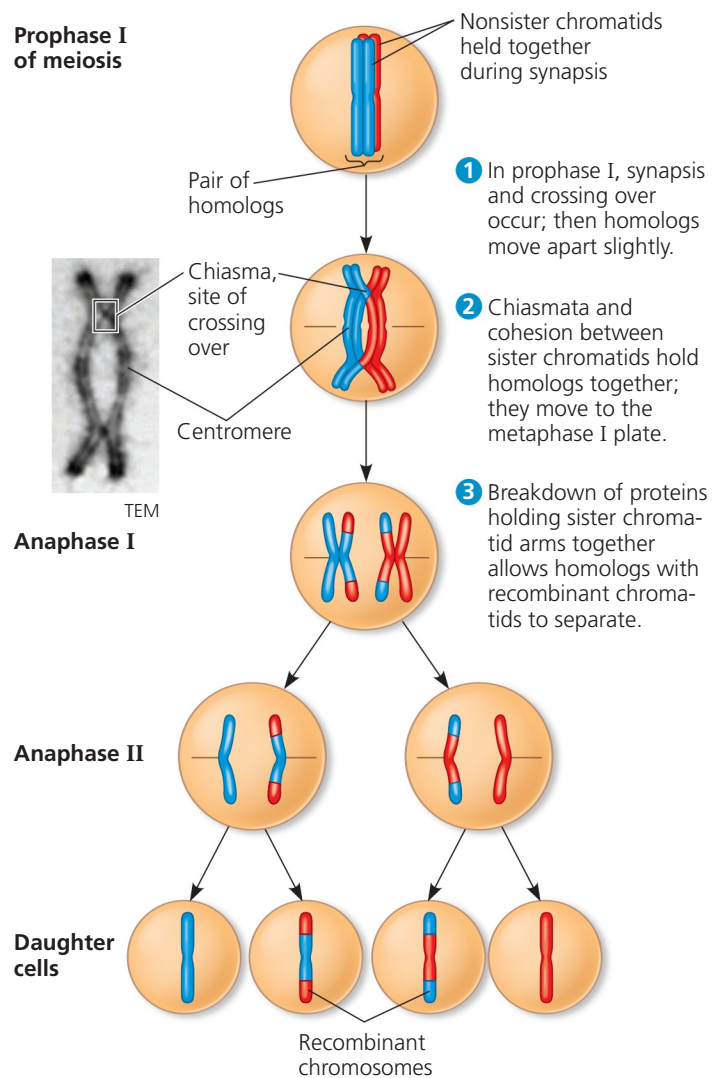
The random nature of fertilization adds to the genetic variation arising from meiosis. In humans, each male and female gamete represents one of about 8.4 million (2^{23}) possible chromosome combinations due to independent assortment. The fusion of a male gamete with a female gamete during fertilization will produce a zygote with any of about 70 trillion ($2^{23} \times 2^{23}$) diploid combinations. If we factor in the variation brought about by crossing over, the number of possibilities is truly astronomical. It may sound trite, but you really *are* unique.

Animation: Genetic Variation from Random Fertilization

The Evolutionary Significance of Genetic Variation Within Populations

EVOLUTION Now that you've learned how new combinations of genes arise among offspring in a sexually reproducing population, how does the genetic variation in a population relate to evolution? Darwin recognized that a population evolves through the differential reproductive success of its variant members. On average, those individuals best suited to the local environment leave the most offspring, thereby transmitting their genes. Thus, natural selection results in the accumulation of genetic variations favored by the environment. As the environment changes, the population may survive if, in each generation, at least some of its members can cope effectively with the new conditions. Mutations are the original source of different alleles, which are then mixed and

▼ Figure 13.12 The results of crossing over during meiosis.



Animation: Genetic Variation from Crossing Over

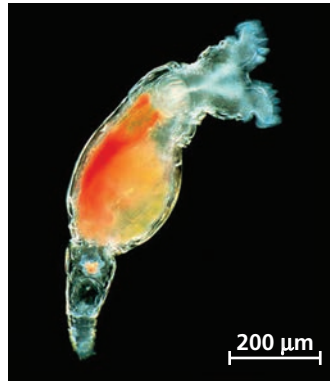
matched during meiosis. New and different combinations of alleles may work better than those that previously prevailed.

In a stable environment, though, sexual reproduction seems as if it would be less advantageous than asexual reproduction, which ensures perpetuation of successful combinations of alleles. Furthermore, sexual reproduction is more expensive energetically than asexual reproduction. In spite of these apparent disadvantages, sexual reproduction is almost universal among animals. Why is this?

The ability of sexual reproduction to generate genetic diversity is the most commonly proposed explanation for the evolutionary persistence of this process. However, consider the unusual case of the bdelloid rotifer (**Figure 13.13**). It appears that this group may not have reproduced sexually for more than 50 million years of their evolutionary history, a model that has been supported by recent analysis of the genetic sequences in its genome. Does this mean that genetic diversity is not advantageous in this species? It turns out that bdelloid rotifers are an exception to the “rule” that sex alone generates

genetic diversity: Bdelloids have mechanisms other than sexual reproduction for generating genetic diversity. For example, they live in environments that can dry up for long periods of time, during which they can enter a state of suspended animation. In this state, their cell membranes may crack in places, allowing entry of DNA from other rotifer species and even from more distantly related species. Evidence suggests that this foreign DNA can become incorporated into the genome of the bdelloid, leading to increased genetic diversity. In fact, the genomic analysis shows that bdelloid rotifers pick up non-bdelloid DNA at a much higher rate than most other species pick up foreign DNA. The conclusion that bdelloid rotifers have developed other ways of achieving genetic diversity supports the idea that genetic diversity is advantageous, but that sexual reproduction is not the only way of generating such diversity.

▼ **Figure 13.13** A bdelloid rotifer, an animal that reproduces only asexually.



In this chapter, we have seen how sexual reproduction greatly increases the genetic variation present in a population. Although Darwin realized that heritable variation is what makes evolution possible, he could not explain why offspring resemble—but are not identical to—their parents. Ironically, Gregor Mendel, a contemporary of Darwin, published a theory of inheritance that helps explain genetic variation, but his discoveries had no impact on biologists until 1900, more than 15 years after Darwin (1809–1882) and Mendel (1822–1884) had died. In the next chapter, you'll learn how Mendel discovered the basic rules governing the inheritance of specific traits.

CONCEPT CHECK 13.4

1. What is the original source of variation among the different alleles of a gene?
2. The diploid number for fruit flies is 8, and the diploid number for grasshoppers is 46. If no crossing over took place, would the genetic variation among offspring from a given pair of parents be greater in fruit flies or grasshoppers? Explain.
3. **WHAT IF? >** If all chromosomes in a gamete were shortened to half their lengths, how would the genetic diversity resulting from crossing over get affected?

For suggested answers, see Appendix A.

13 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 13.1

Offspring acquire genes from parents by inheriting chromosomes (pp. 305–306)

- Each **gene** in an organism's DNA exists at a specific **locus** on a certain chromosome.
- In **asexual reproduction**, a single parent produces genetically identical offspring by mitosis. **Sexual reproduction** combines genes from two parents, leading to genetically diverse offspring.

? Explain why human offspring resemble their parents but are not identical to them.



CONCEPT 13.2

Fertilization and meiosis alternate in sexual life cycles (pp. 306–309)

- Normal human **somatic cells** are **diploid**. They have 46 chromosomes made up of two sets of 23, one set from each parent. Human diploid cells have 22 pairs of **homologs** that are **autosomes**, and one pair of **sex chromosomes**; the latter typically determines whether the person is female (XX) or male (XY).
- In humans, ovaries and testes produce **haploid gametes** by **meiosis**, each gamete containing a single set of 23 chromosomes ($n = 23$). During **fertilization**, an egg and sperm unite, forming a diploid ($2n = 46$) single-celled **zygote**, which develops into a multicellular organism by mitosis.

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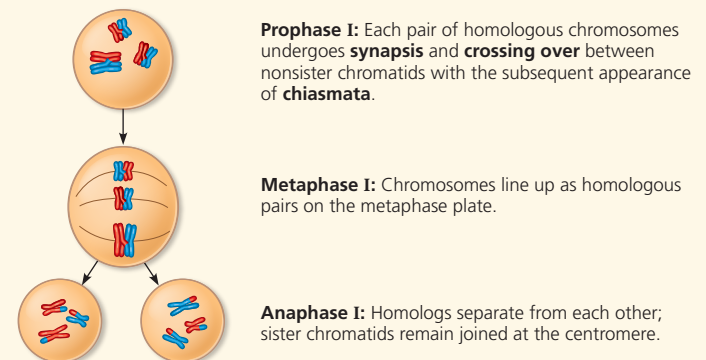
- Sexual life cycles differ in the timing of meiosis relative to fertilization and in the point(s) of the cycle at which a multicellular organism is produced by mitosis.

? Compare the life cycles of animals and plants, mentioning their similarities and differences.

CONCEPT 13.3

Meiosis reduces the number of chromosome sets from diploid to haploid (pp. 309–315)

- The two cell divisions of meiosis, **meiosis I** and **meiosis II**, produce four haploid daughter cells. The number of chromosome sets is reduced from two (diploid) to one (haploid) during meiosis I.
- Meiosis is distinguished from mitosis by three events of meiosis I:



Meiosis II separates the sister chromatids.

- Sister chromatid cohesion and crossing over allow chiasmata to hold homologs together until anaphase I. Cohesins are cleaved along the arms at anaphase I, allowing homologs to separate, and at the centromeres in anaphase II, releasing sister chromatids.

? In prophase I, homologous chromosomes pair up and undergo synapsis and crossing over. Can this also occur during prophase II? Explain.

CONCEPT 13.4

Genetic variation produced in sexual life cycles contributes to evolution (pp. 315–317)

- Three events in sexual reproduction contribute to genetic variation in a population: independent assortment of chromosomes during meiosis I, crossing over during meiosis I, and random fertilization of egg cells by sperm. During crossing over, DNA of nonsister chromatids in a homologous pair is broken and rejoined.
- Genetic variation is the raw material for evolution by natural selection. Mutations are the original source of this variation; recombination of variant genes generates additional genetic diversity.

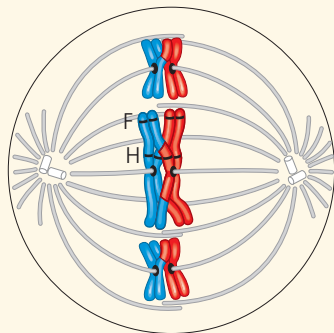
? Explain how three processes unique to sexual reproduction generate a great deal of genetic variation.

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

6. **DRAW IT** The diagram shows a cell in meiosis.
- Label the appropriate structures with these terms: chromosome (label as duplicated or unduplicated), centromere, kinetochore, sister chromatids, nonsister chromatids, homologous pair (use a bracket when labeling), homolog (label each one), chiasma, sister chromatid cohesion, and gene loci, labeling the alleles of the F and H genes.
 - Describe the makeup of a haploid set and a diploid set.
 - Identify the stage of meiosis shown.
7. What would be the consequences of a mutation in the enzyme that cleaves cohesins in the first and second meiotic divisions?
8. **EVOLUTION CONNECTION** Many species can reproduce either asexually or sexually. Explain what you think might be



the evolutionary significance of the switch from asexual to sexual reproduction that occurs in some organisms when the environment becomes unfavorable.

9. **SCIENTIFIC INQUIRY** During the generation of gametes, cell 1 of an organism with three chromosomes undergoes an abnormal separation of one homologous chromosome at the time of the first meiotic division. The second meiotic division is normal. On the other hand, cell 2 undergoes a normal first meiotic division, but one of the daughter cells undergoes an abnormal separation of one sister chromatid at the time of the second meiotic division. What will be the chromosomal distribution in the gametes obtained from cell 1 and cell 2 after meiosis?
10. **WRITE ABOUT A THEME: INFORMATION** The continuity of life is based on heritable information in the form of DNA. In a short essay (100–150 words), explain how chromosome behavior during sexual reproduction in animals ensures perpetuation of parental traits in offspring and, at the same time, genetic variation among offspring.
11. **SYNTHESIZE YOUR KNOWLEDGE**



The Cavendish banana, the world's most popular fruit, is currently threatened by extinction due to a fungus. This banana variety is "triploid" ($3n$, with three sets of chromosomes) and can only reproduce through cloning by cultivators. Given what you know about meiosis, explain how the banana's triploid number accounts for its inability to form normal gametes. Considering genetic diversity, discuss how the absence of sexual reproduction might make this domesticated species vulnerable to infectious agents.

For selected answers, see Appendix A.



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Mendelian Genetics

14

▲ **Figure 14.1** What principles of inheritance did Gregor Mendel discover by breeding pea plants?

KEY CONCEPTS

- 14.1** Mendel used the scientific approach to identify two laws of inheritance
- 14.2** Probability laws govern Mendelian inheritance
- 14.3** Inheritance patterns are often more complex than predicted by simple Mendelian genetics
- 14.4** Many human traits follow Mendelian patterns of inheritance

Drawing from the Deck of Genes

The crowd at a soccer match attests to the marvelous variety and diversity of humankind. Brown, blue, or gray eyes; black, brown, or blond hair—these are just a few examples of heritable variations that we may observe. What principles account for the transmission of such traits from parents to offspring?

The explanation of heredity most widely in favor during the 1800s was the “blending” hypothesis, the idea that genetic material contributed by the two parents mixes just as blue and yellow paints blend to make green. This hypothesis predicts that over many generations, a freely mating population will give rise to a uniform population of individuals, something we don’t see. The blending hypothesis also fails to explain how traits can reappear after they’ve skipped a generation.

An alternative to the blending model is a “particulate” hypothesis of inheritance: the gene idea. In this model, parents pass on discrete heritable units—genes—that retain their separate identities in offspring. An organism’s collection of genes is more like a deck of cards than a pail of paint. Like cards, genes can be shuffled and passed along, generation after generation, in undiluted form.

Modern genetics had its genesis in an abbey garden, where a monk named Gregor Mendel documented a particulate mechanism for inheritance using pea plants (**Figure 14.1**). Mendel developed his theory of inheritance several decades



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Get Ready for This Chapter

◀ **Mendel (third from right, holding a sprig of fuchsia) with his fellow monks.**

before chromosomes were observed under the microscope and the significance of their behavior during mitosis or meiosis was understood. In this chapter, we'll step into Mendel's garden to re-create his experiments and explain how he arrived at his theory of inheritance. We'll also explore inheritance patterns more complex than those observed by Mendel in garden peas. Finally, we will see how the Mendelian model applies to the inheritance of human variations, including hereditary disorders such as sickle-cell disease.

CONCEPT 14.1

Mendel used the scientific approach to identify two laws of inheritance

Mendel discovered the basic principles of heredity by breeding garden peas in carefully planned experiments. As we retrace his work, you will recognize the key elements of the scientific process that were introduced in Chapter 1.

Mendel's Experimental, Quantitative Approach

Mendel grew up on his parents' small farm in a region of Austria that is now part of the Czech Republic. In this agricultural area, Mendel and the other children received agricultural training in school along with their basic education. As an adolescent, Mendel overcame financial hardship and illness to excel in high school and, later, at the Olmutz Philosophical Institute.

In 1843, at the age of 21, Mendel entered an Augustinian monastery, a reasonable choice at that time for someone who valued the life of the mind. He considered becoming a teacher but failed the necessary examination. In 1851, he left the monastery to pursue two years of study in physics and chemistry at the University of Vienna. These were very important years for Mendel's development as a scientist, in large part due to the strong influence of two professors. One was the physicist Christian Doppler, who encouraged his students to learn science through experimentation and trained Mendel to use mathematics to help explain natural phenomena. The other was a botanist named Franz Unger, who aroused Mendel's interest in the causes of variation in plants.

After attending the university, Mendel returned to the monastery and was assigned to teach at a local school, where several other instructors were enthusiastic about scientific research. In addition, his fellow monks shared a long-standing fascination with the breeding of plants. Around 1857, Mendel began breeding garden peas in the abbey garden to study inheritance. Although the question of heredity had long been a focus of curiosity at the monastery, Mendel's fresh approach allowed him to deduce principles that had remained elusive to others.

One reason Mendel probably chose to work with peas is that there are many varieties. For example, one variety has

purple flowers, while another variety has white flowers. A heritable feature that varies among individuals, such as flower color, is called a **character**. Each variant for a character, such as purple or white color for flowers, is called a **trait**.

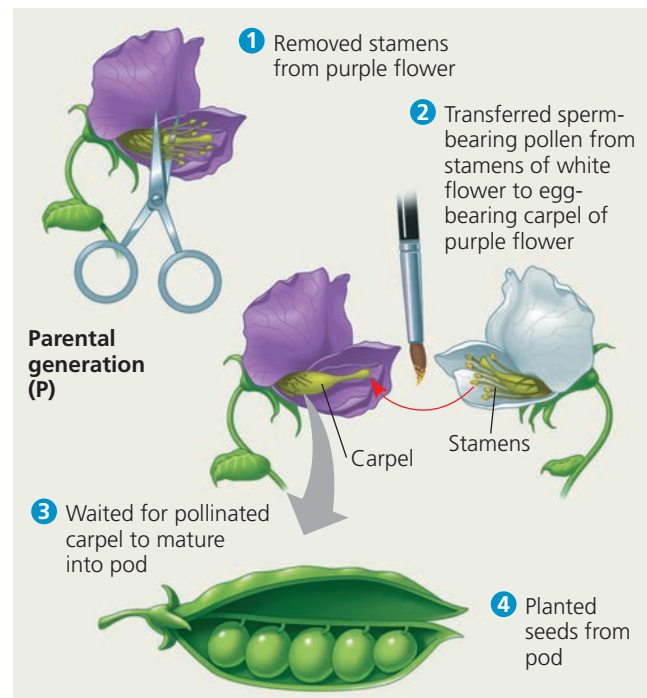
Other advantages of using peas are their short generation time and the large number of offspring from each mating. Furthermore, Mendel could strictly control mating between plants (**Figure 14.2**). Each pea flower has both pollen-producing organs (stamens) and an egg-bearing organ (carpel). In nature, pea plants usually self-fertilize: Pollen grains from the stamens land on the carpel of the same flower, and sperm released

✓ Figure 14.2

Research Method Crossing Pea Plants

Application By crossing (mating) two true-breeding varieties of an organism, scientists can study patterns of inheritance. In this example, Mendel crossed pea plants that varied in flower color.

Technique



Results When pollen from a white flower was transferred to a purple flower, the first-generation hybrids all had purple flowers. The result was the same for the reciprocal cross, which involved the transfer of pollen from purple flowers to white flowers.



from the pollen grains fertilize eggs present in the carpel.* To achieve cross-pollination of two plants, Mendel removed the immature stamens of a plant before they produced pollen and then dusted pollen from another plant onto the altered flowers (see Figure 14.2). Each resulting zygote then developed into a plant embryo encased in a seed (pea). His method allowed Mendel to always be sure of the parentage of new seeds.

Mendel chose to track only those characters that occurred in two distinct, alternative forms, such as purple or white flower color. He also made sure that he started his experiments with varieties that were **true-breeding**—that is, over many generations of self-pollination, these plants had produced only the same variety as the parent plant. For example, a plant with purple flowers is true-breeding if the seeds produced by self-pollination in successive generations all give rise to plants that also have purple flowers.

In a typical breeding experiment, Mendel cross-pollinated two contrasting, true-breeding pea varieties—for example, purple-flowered plants and white-flowered plants (see Figure 14.2). This mating, or *crossing*, of two true-breeding varieties is called **hybridization**. The true-breeding parents are referred to as the **P generation** (parental generation), and their hybrid offspring are the **F₁ generation** (first filial generation, the word *filial* from the Latin word for “son”). Allowing these F₁ hybrids to self-pollinate (or to cross-pollinate with other F₁ hybrids) produces an **F₂ generation** (second filial generation). Mendel usually followed traits for at least the P, F₁, and F₂ generations. Had Mendel stopped his experiments with the F₁ generation, the basic patterns of inheritance would have eluded him. Mendel’s quantitative analysis of the F₂ plants from thousands of genetic crosses like these allowed him to deduce two fundamental principles of heredity, now called the law of segregation and the law of independent assortment.

The Law of Segregation

If the blending model of inheritance were correct, the F₁ hybrids from a cross between purple-flowered and white-flowered pea plants would have pale purple flowers, a trait intermediate between those of the P generation. Notice in Figure 14.2 that the experiment produced a very different result: All the F₁ offspring had flowers of the same color as the purple-flowered parents. What happened to the white-flowered plants’ genetic contribution to the hybrids? If it were lost, then the F₁ plants could produce only purple-flowered offspring in the F₂ generation. But when Mendel allowed the F₁ plants to self- or cross-pollinate and planted their seeds, the white-flower trait reappeared in the F₂ generation.

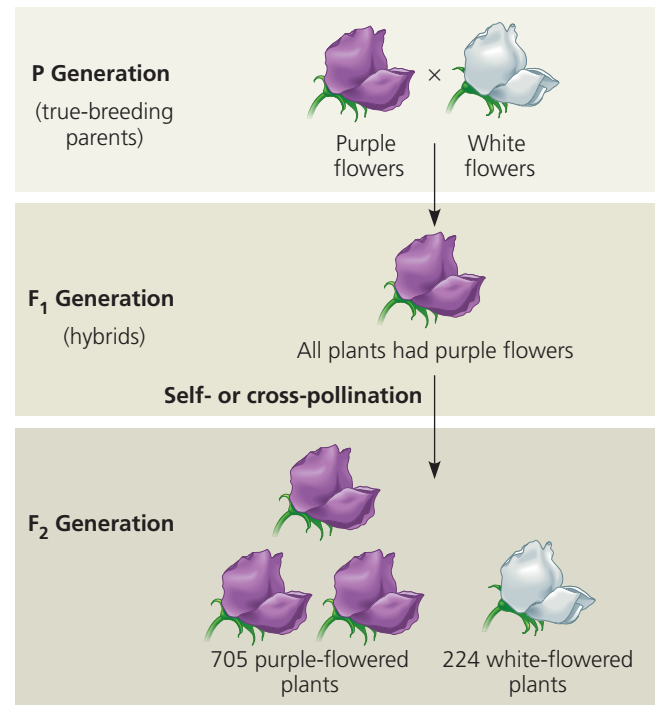
*As you learned in Figure 13.6b, meiosis in plants produces spores, not gametes. In flowering plants like the pea, each spore develops into a microscopic haploid gametophyte that contains only a few cells and is located on the parent plant. The gametophyte produces sperm, in pollen grains, and eggs, in the carpel. For simplicity, we will not include the gametophyte stage in our discussion of fertilization in plants.

Mendel used very large sample sizes and kept accurate records of his results: 705 of the F₂ plants had purple flowers, and 224 had white flowers. These data fit a ratio of approximately three purple to one white (**Figure 14.3**). Mendel reasoned that the heritable factor for white flowers did not disappear in the F₁ plants but was somehow hidden, or masked, when the purple-flower factor was present. In Mendel’s terminology, purple flower color is a *dominant* trait, and white flower color is a *recessive* trait. The reappearance of white-flowered plants in the F₂ generation was evidence

✓ Figure 14.3

Inquiry When F₁ hybrid pea plants self- or cross-pollinate, which traits appear in the F₂ generation?

Experiment Mendel crossed true-breeding purple-flowered plants and white-flowered plants (crosses are symbolized by ×). The resulting F₁ hybrids were allowed to self-pollinate or were cross-pollinated with other F₁ hybrids. The F₂ generation plants were then observed for flower color.



Results Both purple-flowered and white-flowered plants appeared in the F₂ generation, in a ratio of approximately 3:1.

Conclusion The “heritable factor” for the recessive trait (white flowers) had not been destroyed, deleted, or “blended” in the F₁ generation but was merely masked by the presence of the factor for purple flowers, which is the dominant trait.

Data from G. Mendel, Experiments in plant hybridization, *Proceedings of the Natural History Society of Brunn* 4:3–47 (1866).

WHAT IF? > If you mated two purple-flowered plants from the P generation, what ratio of traits would you expect to observe in the offspring? Explain. What might Mendel have concluded if he stopped his experiment after the F₁ generation?

that the heritable factor causing white flowers had not been diluted or destroyed by coexisting with the purple-flower factor in the F₁ hybrids. Instead, it had been hidden when in the presence of the purple-flower factor.

Mendel observed the same pattern of inheritance in six other characters, each represented by two distinctly different traits (**Table 14.1**). For example, when Mendel crossed a true-breeding variety that produced smooth, round pea seeds with one that produced wrinkled seeds, all the F₁ hybrids produced round seeds; this is the dominant trait for seed shape. In the F₂ generation, approximately 75% of the seeds were round and 25% were wrinkled—a 3:1 ratio, as in Figure 14.3. Now let’s see how Mendel deduced the law of segregation from his experimental results. In the discussion that follows, we will use modern terms instead of some of the terms used by Mendel. (For example, we’ll use “gene” instead of Mendel’s “heritable factor.”)

Table 14.1 The Results of Mendel’s F ₁ Crosses for Seven Characters in Pea Plants					
Character	Dominant Trait	×	Recessive Trait	F ₂ Generation Dominant:Recessive	Ratio
Flower color	Purple 	×	White 	705:224	3.15:1
Seed color	Yellow 	×	Green 	6,022:2,001	3.01:1
Seed shape	Round 	×	Wrinkled 	5,474:1,850	2.96:1
Pod color	Green 	×	Yellow 	428:152	2.82:1
Pod shape	Inflated 	×	Constricted 	882:299	2.95:1
Flower position	Axial 	×	Terminal 	651:207	3.14:1
Stem length	Tall 	×	Dwarf 	787:277	2.84:1

Mendel’s Model

Mendel developed a model to explain the 3:1 inheritance pattern that he consistently observed among the F₂ offspring in his pea experiments. We describe four related concepts making up this model, the fourth of which is the law of segregation.

First, *alternative versions of genes account for variations in inherited characters*. The gene for flower color in pea plants, for example, exists in two versions, one for purple flowers and the other for white flowers. These alternative versions of a gene are called **alleles**. Today, we can relate this concept to chromosomes and DNA. As shown in **Figure 14.4**, each gene is a sequence of nucleotides at a specific place, or locus, along a particular chromosome. The DNA at that locus, however, can vary slightly in its nucleotide sequence. This variation in information content can affect the function of the encoded protein and thus an inherited character of the organism. The purple-flower allele and the white-flower allele are two DNA sequence variations possible at the flower-color locus on a pea plant’s chromosomes. The purple-flower allele sequence allows synthesis of purple pigment, and the white-flower allele sequence does not.

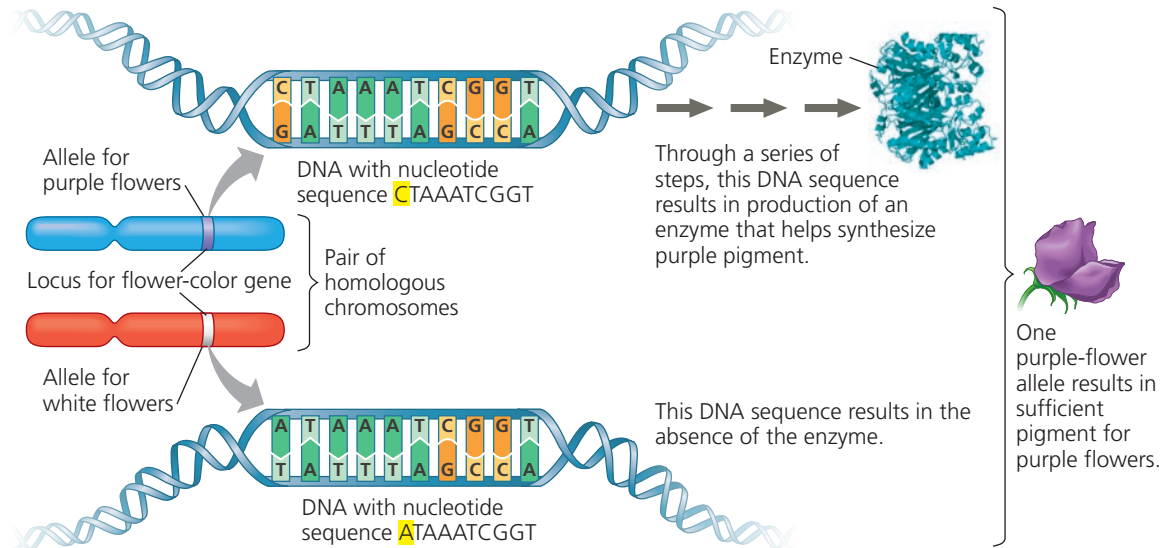
Second, *for each character, an organism inherits two copies (that is, two alleles) of a gene, one from each parent*. Remarkably, Mendel made this deduction without knowing about the role, or even the existence, of chromosomes. Each somatic cell in a diploid organism has two sets of chromosomes, one set inherited from each parent (see Figure 13.4). Thus, a genetic locus is actually represented twice in a diploid cell, once on each homolog of a specific pair of chromosomes. The two alleles at a particular locus may be identical, as in the true-breeding plants of Mendel’s P generation. Or the alleles may differ, as in the F₁ hybrids (see Figure 14.4).

Third, *if the two alleles at a locus differ, then one, the **dominant allele**, determines the organism’s appearance; the other, the **recessive allele**, has no noticeable effect on the organism’s appearance*. Accordingly, Mendel’s F₁ plants had purple flowers because the allele for that trait is dominant and the allele for white flowers is recessive.

The fourth and final part of Mendel’s model, the **law of segregation**, states that *the two alleles for a heritable character segregate (separate from each other) during gamete formation and end up in different gametes*. Thus, an egg or a sperm gets only one of the two alleles that are present in the somatic cells of the organism making the gamete. In terms of chromosomes, this segregation corresponds to the distribution of copies of the two members of a pair of homologous chromosomes to different gametes in meiosis (see Figure 13.7). Note that if an organism has identical alleles for a particular character then that allele is present in all gametes. Because it is the only allele that can be passed on to offspring, the offspring always look like their parents; this explains why these plants are true-breeding. But if different alleles are present, as in the F₁ hybrids, then 50% of the gametes receive the dominant allele and 50% receive the recessive allele.

➤ **Figure 14.4 Alleles, alternative versions of a gene.**

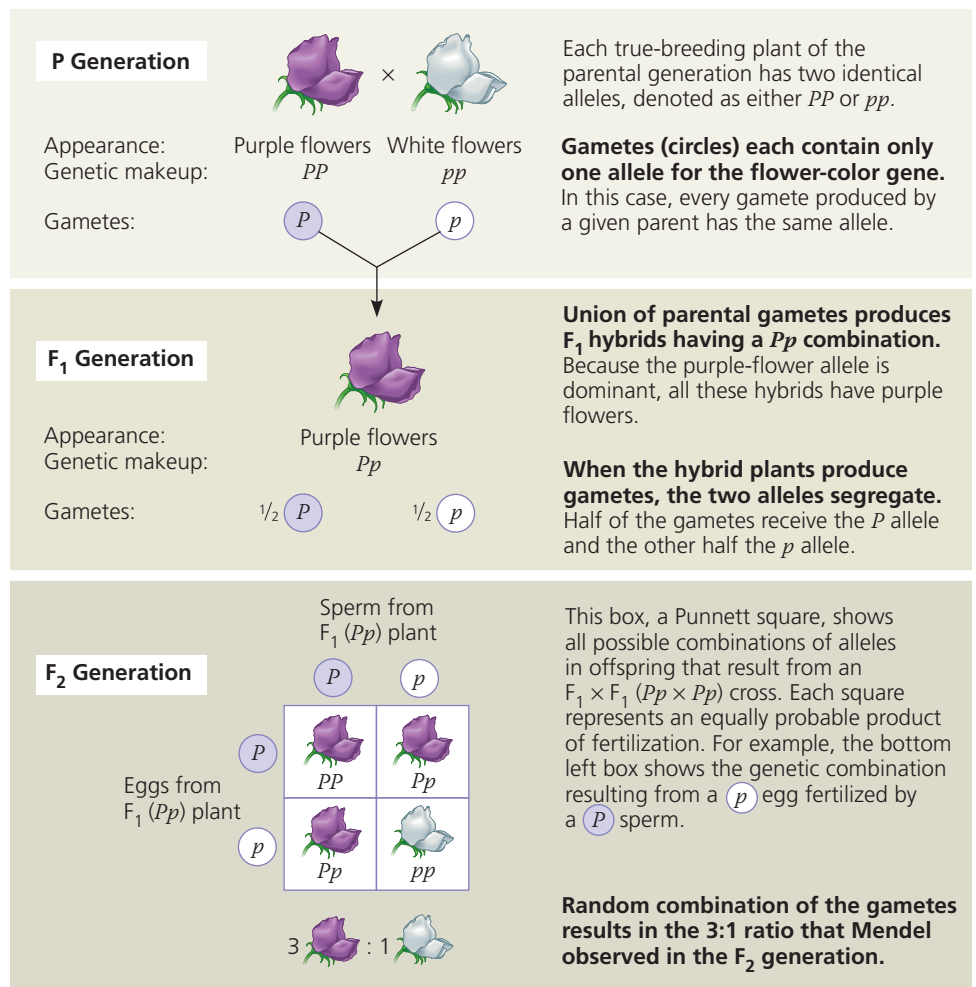
This diagram shows a pair of homologous chromosomes in an F_1 hybrid pea plant, with the actual DNA sequence from the flower-color allele of each chromosome. The paternally inherited chromosome (blue) has an allele for purple flowers, which codes for a protein that indirectly controls synthesis of purple pigment. The maternally inherited chromosome (red) has an allele for white flowers, which results in no functional protein being made.



Does Mendel's segregation model account for the 3:1 ratio he observed in the F_2 generation of his numerous crosses? For the flower-color character, the model predicts that the two different alleles present in an F_1 individual will segregate into gametes such that half the gametes will have the purple-flower allele and half will have the white-flower allele. During self-pollination, gametes of each class unite randomly. An egg with a purple-flower allele has an equal chance of being fertilized by a sperm with a purple-flower allele or by one with a white-flower allele. Since the same is true for an egg with a white-flower allele, there are four equally likely combinations of sperm and egg. **Figure 14.5** illustrates these combinations using a **Punnett square**, a handy diagrammatic device for predicting the allele composition of offspring from a cross between individuals of known genetic makeup. Notice that we use a capital letter to symbolize a dominant allele and a lowercase letter for a recessive allele. In our example, P is the purple-flower allele, and p is the white-flower allele; it is often useful as well to be able to refer to the gene itself as the P/p gene.

In the F_2 offspring, what color will the flowers be? One-fourth of the plants have inherited two purple-flower alleles; clearly, these plants will have

▼ **Figure 14.5 Mendel's law of segregation.** This diagram shows the genetic makeup of the generations in Figure 14.3. It illustrates Mendel's model for inheritance of the alleles of a single gene. Each plant has two alleles for the gene controlling flower color, one allele inherited from each of the plant's parents. To construct a Punnett square that predicts the F_2 generation offspring, we list all the possible gametes from one parent (here, the F_1 female) along the left side of the square and all the possible gametes from the other parent (here, the F_1 male) along the top. The boxes represent the offspring resulting from all the possible unions of male and female gametes.



Animation: Mendel's Cross of One Character: Flower Color
Animation: Simplified Crosses of One Character in Humans
Animation: Cross of One Character in "MendAliens"

purple flowers. One-half of the F_2 offspring have inherited one purple-flower allele and one white-flower allele; these plants will also have purple flowers, the dominant trait. Finally, one-fourth of the F_2 plants have inherited two white-flower alleles and will express the recessive trait. Thus, Mendel's model accounts for the 3:1 ratio of traits that he observed in the F_2 generation.

Useful Genetic Vocabulary

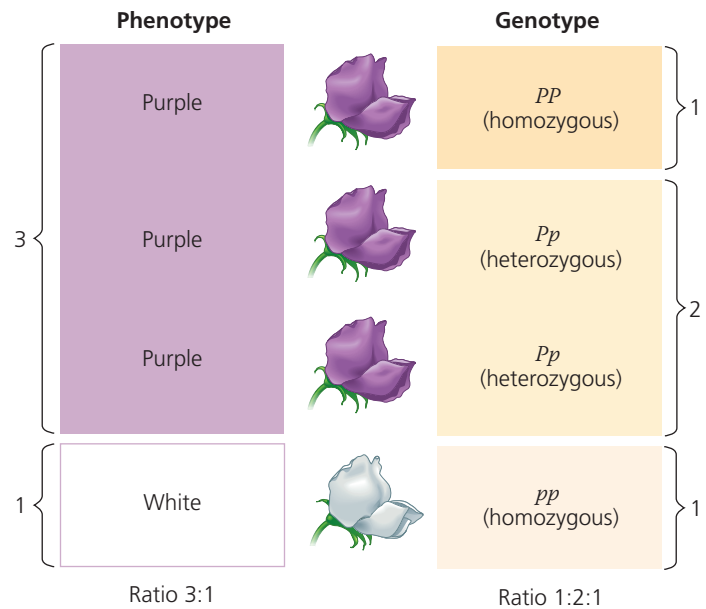
An organism that has a pair of identical alleles for a gene encoding a character is called a **homozygote** and is said to be **homozygous** for that gene. In the parental generation in Figure 14.5, the purple-flowered pea plant is homozygous for the dominant allele (PP), while the white plant is homozygous for the recessive allele (pp). Homozygous plants “breed true” because all of their gametes contain the same allele—either P or p in this example. If we cross dominant homozygotes with recessive homozygotes, every offspring will have two different alleles— Pp in the case of the F_1 hybrids of our flower-color experiment (see Figure 14.5). An organism that has two different alleles for a gene is called a **heterozygote** and is said to be **heterozygous** for that gene. Unlike homozygotes, heterozygotes produce gametes with different alleles, so they are not true-breeding. For example, P - and p -containing gametes are both produced by our F_1 hybrids. Self-pollination of the F_1 hybrids thus produces both purple-flowered and white-flowered offspring.

Because of the different effects of dominant and recessive alleles, an organism's traits do not always reveal its genetic composition. Therefore, we distinguish between an organism's appearance or observable traits, called its **phenotype**, and its genetic makeup, its **genotype**. As shown in Figure 14.5 for the case of flower color in pea plants, PP and Pp plants have the same phenotype (purple flowers) but different genotypes. **Figure 14.6** reviews these terms. Note that the term *phenotype* refers to physiological traits as well as traits that relate directly to appearance. For example, one pea variety lacks the normal ability to self-pollinate, which is a phenotypic trait (called non-self-pollination).

The Testcross

Given a purple-flowered pea plant, we cannot tell if it is homozygous (PP) or heterozygous (Pp) because both genotypes result in the same purple phenotype. To determine the genotype, we can cross this plant with a white-flowered plant (pp), which will make only gametes with the recessive allele (p). The allele in the gamete contributed by the purple-flowered plant of unknown genotype will therefore determine the appearance of the offspring (**Figure 14.7**). If all the offspring of the cross have purple flowers, then the purple-flowered mystery plant must be homozygous for the dominant allele, because a $PP \times pp$ cross produces all Pp offspring. But if both the purple and the white phenotypes appear among the offspring, then

the purple-flowered parent must be heterozygous. The offspring of a $Pp \times pp$ cross will be expected to have a 1:1 phenotypic ratio. Breeding an organism of unknown genotype with a recessive homozygote is called a **testcross** because it can reveal the genotype of that organism. The testcross was devised by Mendel and continues to be used by geneticists.



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The Law of Independent Assortment

Mendel derived the law of segregation from experiments in which he followed only a *single* character, such as flower color. All the F_1 progeny produced in his crosses of true-breeding parents were **monohybrids**, meaning that they were heterozygous for the one particular character being followed in the cross. We refer to a cross between such heterozygotes as a **monohybrid cross**.

Mendel worked out the second law of inheritance by following *two* characters at the same time, such as seed color and seed shape. Seeds (peas) may be either yellow or green. They also may be either round (smooth) or wrinkled. From single-character crosses, Mendel knew that the allele for yellow seeds is dominant (Y), and the allele for green seeds is recessive (y). For the seed-shape character, the allele for round is dominant (R), and the allele for wrinkled is recessive (r).

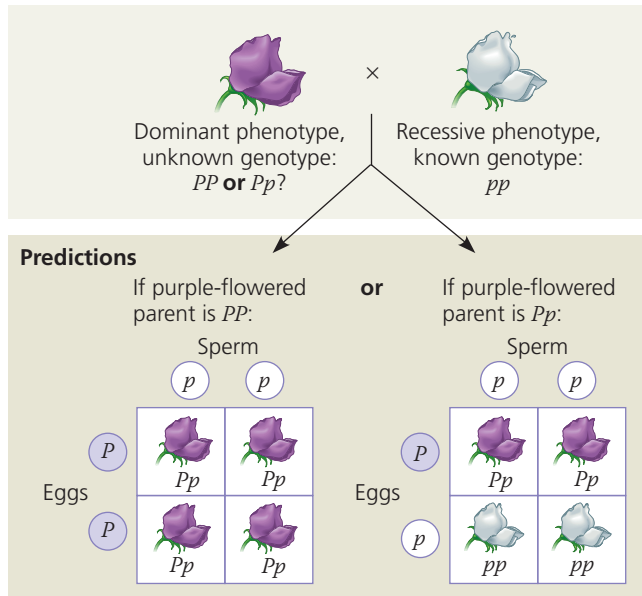
Imagine crossing two true-breeding pea varieties that differ in *both* of these characters—a cross between a plant with yellow-round seeds ($YYRR$) and a plant with green-wrinkled seeds ($yyrr$). The F_1 plants will be **dihybrids**, individuals heterozygous for the two characters being followed in the cross ($YyRr$). But are these two characters transmitted from parents

▼ Figure 14.7

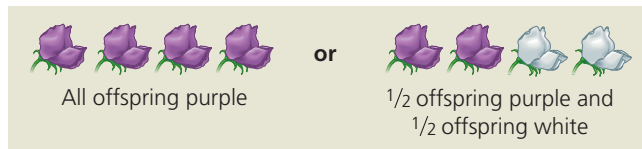
Research Method The Testcross

Application An organism that shows a dominant trait in its phenotype, such as purple flowers in pea plants, can be either homozygous for the dominant allele or heterozygous. To determine the organism's genotype, geneticists can perform a testcross.

Technique In a testcross, the individual with the unknown genotype is crossed with a homozygous individual expressing the recessive trait (white flowers in this example), and Punnett squares are used to predict the possible outcomes.



Results Matching the results to either prediction identifies the unknown parental genotype (either PP or Pp in this example). In this testcross, we transferred pollen from a white-flowered plant to the carpels of a purple-flowered plant; the opposite (reciprocal) cross would have led to the same results.



Animation: Testcross in "MendAliens"

to offspring as a package? That is, will the Y and R alleles always stay together, generation after generation? Or are seed color and seed shape inherited independently? **Figure 14.8** shows how a **dihybrid cross**, a cross between F_1 dihybrids, can determine which of these two hypotheses is correct.

The F_1 plants, of genotype $YyRr$, exhibit both dominant phenotypes, yellow seeds with round shapes, no matter which hypothesis is correct. The key step in the experiment is to see what happens when F_1 plants self-pollinate and produce F_2 offspring. If the hybrids must transmit their alleles in the same combinations in which the alleles were inherited from the P generation, then the F_1 hybrids will produce only

two classes of gametes: YR and yr . As shown on the left side of Figure 14.8, this "dependent assortment" hypothesis predicts that the phenotypic ratio of the F_2 generation will be 3:1, just as in a monohybrid cross:



The alternative hypothesis is that the two pairs of alleles segregate independently of each other. In other words, genes are packaged into gametes in all possible allelic combinations, as long as each gamete has one allele for each gene (see Figure 13.11). In our example, an F_1 plant will produce four classes of gametes in equal quantities: YR , Yr , yR , and yr . If sperm of the four classes fertilize eggs of the four classes, there will be 16 (4×4) equally probable ways in which the alleles can combine in the F_2 generation, as shown on the right side of Figure 14.8. These combinations result in four phenotypic categories with a ratio of 9:3:3:1 (nine yellow round to three green round to three yellow wrinkled to one green wrinkled):



When Mendel did the experiment and classified the F_2 offspring, his results were close to the predicted 9:3:3:1 phenotypic ratio, supporting the hypothesis that the alleles for one gene—controlling seed color, for example—segregate into gametes independently of the alleles of any other gene, such as seed shape.

Mendel tested his seven pea characters in various dihybrid combinations and always observed a 9:3:3:1 phenotypic ratio in the F_2 generation. Is this consistent with the 3:1 phenotypic ratio seen for the monohybrid cross shown in Figure 14.5? To explore this question, count the number of yellow and green peas, ignoring shape, and calculate the ratio. The results of Mendel's dihybrid experiments are the basis for what we now call the **law of independent assortment**, which states that *two or more genes assort independently—that is, each pair of alleles segregates independently of any other pair of alleles—during gamete formation*.

This law applies only to genes (allele pairs) located on different chromosomes (that is, on chromosomes that are not homologous) or, alternatively, to genes that are very far apart on the same chromosome. (This will be explained in Concept 15.3, along with the more complex inheritance patterns of genes located near each other, alleles of which tend to be inherited together.) All the pea characters Mendel chose for analysis were controlled by genes on different chromosomes or were far apart on the same chromosome; this situation greatly simplified interpretation of his multicharacter pea crosses. All the examples we consider in the rest of this chapter involve genes located on different chromosomes.

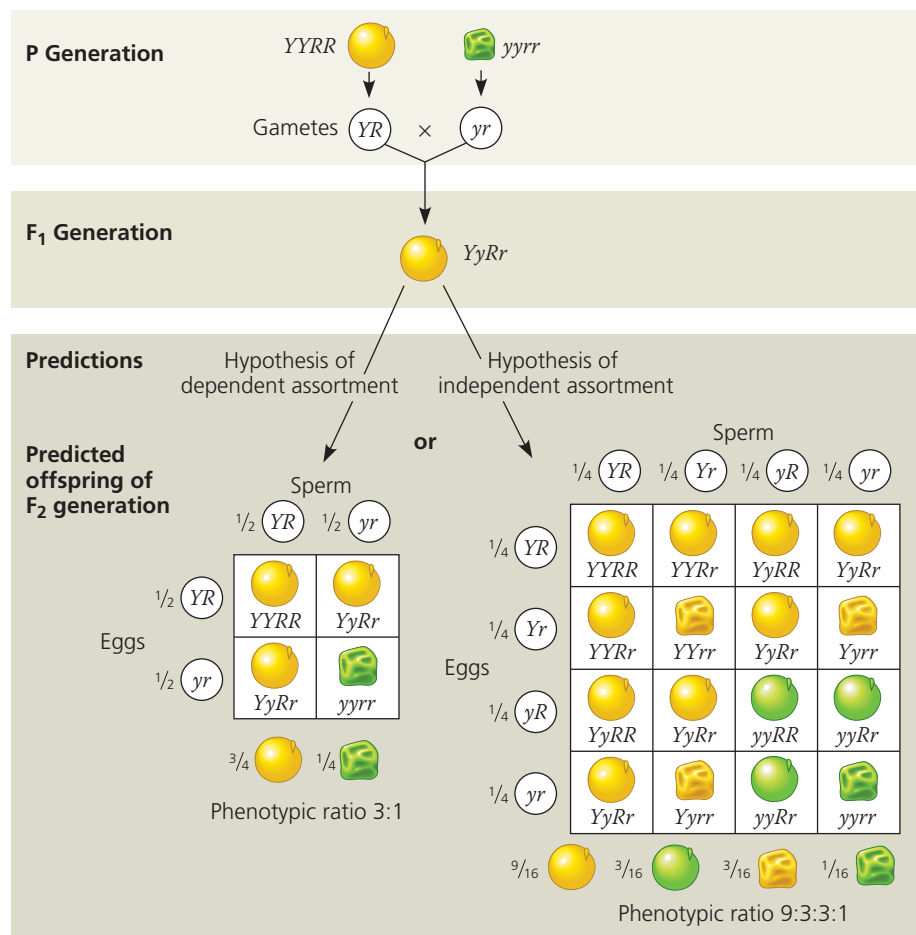


Animation: Independent Assortment

▼ Figure 14.8

Inquiry Do the alleles for one character segregate into gametes dependently or independently of the alleles for a different character?

Experiment To follow the characters of seed color and seed shape through the F_2 generation, Mendel crossed a true-breeding plant with yellow round seeds with a true-breeding plant with green wrinkled seeds, producing dihybrid F_1 plants. Self-pollination of the F_1 dihybrids produced the F_2 generation. The two hypotheses (dependent and independent “assortment” of the two genes) predict different phenotypic ratios.



Results

315 Yellow Round 108 Green Round 101 Yellow Wrinkled 32 Green Wrinkled Phenotypic ratio approximately 9:3:3:1

Conclusion The results support the hypothesis of independent assortment, the only one that predicts two newly observed phenotypes: green round seeds and yellow wrinkled seeds (see the right-hand Punnett square). The alleles for each gene segregate independently of those of the other, and the two genes are said to assort independently.

Data from G. Mendel, Experiments in plant hybridization, *Proceedings of the Natural History Society of Brunn* 4:3–47 (1866).

WHAT IF? > Suppose Mendel had transferred pollen from an F_1 plant to the carpel of a plant that was homozygous recessive for both genes. Set up the cross and draw Punnett squares that predict the offspring for both hypotheses. Would this cross have supported the hypothesis of independent assortment equally well?

Animation: Mendel's Cross of Two Characters: Seed Shape and Seed Color
Animation: A Simplified Cross of Two Characters in Humans
Animation: Crosses of Two Characters in “MendAliens”

CONCEPT CHECK 14.1

- DRAW IT >** Pea plants heterozygous for flower position and stem length ($AaTt$) are allowed to self-pollinate, and 400 of the resulting seeds are planted. Draw a Punnett square for this cross. How many offspring would be predicted to have terminal flowers and be dwarf? (See Table 14.1.)
- WHAT IF? >** List all gametes that could be made by a pea plant heterozygous for seed color, seed shape, and pod shape ($YyRrIi$; see Table 14.1). How large a Punnett square would you need to draw to predict the offspring of a self-pollination of this “trihybrid”?
- MAKE CONNECTIONS >** In some pea plant crosses, the plants are self-pollinated. Is self-pollination considered asexual or sexual reproduction? Explain. (See Concept 13.1.)

For suggested answers, see Appendix A.

CONCEPT 14.2

Probability laws govern Mendelian inheritance

Mendel’s laws of segregation and independent assortment reflect the same rules of probability that apply to tossing coins, rolling dice, and drawing cards from a deck. The probability scale ranges from 0 to 1. An event that is certain to occur has a probability of 1, while an event that is certain *not* to occur has a probability of 0. With a coin that has heads on both sides, the probability of tossing heads is 1, and the probability of tossing tails is 0. With a normal coin, the chance of tossing heads is $\frac{1}{2}$, and the chance of tossing tails is $\frac{1}{2}$. The probability of drawing the ace of spades from a 52-card deck is $\frac{1}{52}$. The probabilities of all possible outcomes for an event must add up to 1. With a deck of cards, the chance of picking a card other than the ace of spades is $\frac{51}{52}$.

Tossing a coin illustrates an important lesson about probability. For every toss, the probability of heads is $\frac{1}{2}$. The outcome of any particular toss is unaffected by what has happened on previous trials. We refer to phenomena such as coin tosses as independent events. Each toss of a coin, whether done sequentially

with one coin or simultaneously with many, is independent of every other toss. And like two separate coin tosses, the alleles of one gene segregate into gametes independently of another gene's alleles (the law of independent assortment). We'll now look at two basic rules of probability that help us predict the outcome of the fusion of such gametes in simple monohybrid crosses and more complicated crosses as well.

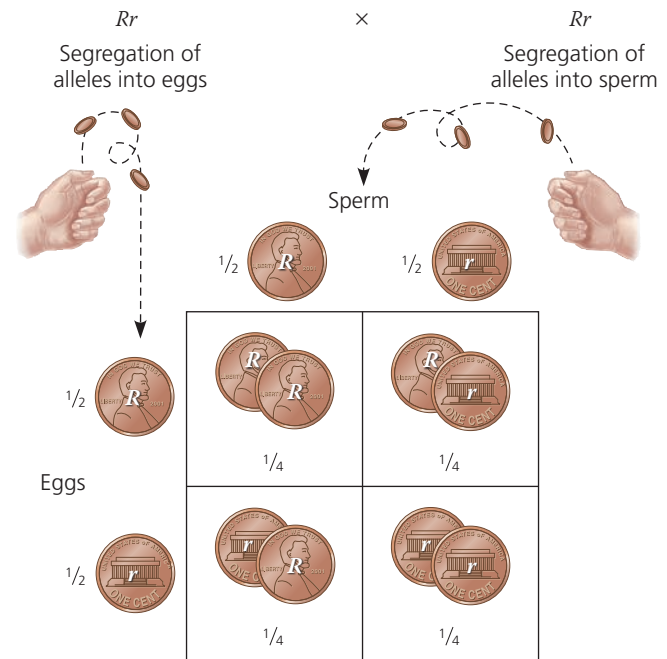
The Multiplication and Addition Rules Applied to Monohybrid Crosses

How do we determine the probability that two or more independent events will occur together in some specific combination? For example, what is the chance that two coins tossed simultaneously will both land heads up? The **multiplication rule** states that to determine this probability, we multiply the probability of one event (one coin coming up heads) by the probability of the other event (the other coin coming up heads). By the multiplication rule, then, the probability that both coins will land heads up is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$.

We can apply the same reasoning to an F_1 monohybrid cross. With seed shape in pea plants as the heritable character, the genotype of F_1 plants is Rr . Segregation in a heterozygous plant is like flipping a coin in terms of calculating the probability of each outcome: Each egg produced has a $\frac{1}{2}$ chance of carrying the dominant allele (R) and a $\frac{1}{2}$ chance of carrying the recessive allele (r). The same odds apply to each sperm cell produced. For a particular F_2 plant to have wrinkled seeds, the recessive trait, both the egg and the sperm that come together must carry the r allele. The probability that an r allele will be present in both gametes at fertilization is found by multiplying $\frac{1}{2}$ (the probability that the egg will have an r) $\times$ $\frac{1}{2}$ (the probability that the sperm will have an r). Thus, the multiplication rule tells us that the probability of an F_2 plant having wrinkled seeds (rr) is $\frac{1}{4}$ (Figure 14.9). Likewise, the probability of an F_2 plant carrying both dominant alleles for seed shape (RR) is $\frac{1}{4}$.

To figure out the probability that an F_2 plant from a monohybrid cross will be heterozygous rather than homozygous, we need to invoke a second rule. Notice in Figure 14.9 that the dominant allele can come from the egg and the recessive allele from the sperm, or vice versa. That is, F_1 gametes can combine to produce Rr offspring in two *mutually exclusive* ways: For any particular heterozygous F_2 plant, the dominant allele can come from the egg *or* the sperm, but not from both. According to the **addition rule**, the probability that any one of two or more mutually exclusive events will occur is calculated by adding their individual probabilities. As we have just seen, the multiplication rule gives us the individual probabilities that we will now add together. The probability for one possible way of obtaining an F_2 heterozygote—the dominant allele from the egg and the recessive allele from the sperm—is $\frac{1}{4}$. The probability for the other possible way—the recessive allele from the egg and the dominant allele

Figure 14.9 Segregation of alleles and fertilization as chance events. When a heterozygote (Rr) forms gametes, whether a particular gamete ends up with an R or an r is like the toss of a coin. We can determine the probability for any genotype among the offspring of two heterozygotes by multiplying together the individual probabilities of an egg and sperm having a particular allele (R or r in this example).



from the sperm—is also $\frac{1}{4}$ (see Figure 14.9). Using the rule of addition, then, we can calculate the probability of an F_2 heterozygote as $\frac{1}{4} + \frac{1}{4} = \frac{1}{2}$.

Solving Complex Genetics Problems with the Rules of Probability

We can also apply the rules of probability to predict the outcome of crosses involving multiple characters. Recall that each allelic pair segregates independently during gamete formation (the law of independent assortment). Thus, a dihybrid or other multicharacter cross is equivalent to two or more independent monohybrid crosses occurring simultaneously. By applying what we have learned about monohybrid crosses, we can determine the probability of specific genotypes occurring in the F_2 generation without having to construct unwieldy Punnett squares.

Consider the dihybrid cross between $YyRr$ heterozygotes shown in Figure 14.8. We will focus first on the seed-color character. For a monohybrid cross of Yy plants, we can use a simple Punnett square to determine that the probabilities of the offspring genotypes are $\frac{1}{4}$ for YY , $\frac{1}{2}$ for Yy , and $\frac{1}{4}$ for yy . We can draw a second Punnett square to determine that the same probabilities apply to the offspring genotypes for seed shape: $\frac{1}{4}$ RR , $\frac{1}{2}$ Rr , and $\frac{1}{4}$ rr . Knowing these probabilities, we can simply use the multiplication rule to determine the probability of each of the genotypes in the F_2 generation.

To give two examples, the calculations for finding the probabilities of two of the possible F_2 genotypes ($YYRR$ and $YyRR$) are shown below:

$$\text{Probability of } YYRR = \frac{1}{4}(\text{probability of } YY) \times \frac{1}{4}(RR) = \frac{1}{16}$$

$$\text{Probability of } YyRR = \frac{1}{2}(Yy) \times \frac{1}{4}(RR) = \frac{1}{8}$$

The $YYRR$ genotype corresponds to the upper left box in the larger Punnett square in Figure 14.8 (one box = $\frac{1}{16}$). Looking closely at the larger Punnett square in Figure 14.8, you will see that 2 of the 16 boxes ($\frac{1}{8}$) correspond to the $YyRR$ genotype.

Now let's see how we can combine the multiplication and addition rules to solve even more complex problems in Mendelian genetics. Imagine a cross of two pea varieties in which we track the inheritance of three characters. Let's cross a trihybrid with purple flowers and yellow, round seeds (heterozygous for all three genes) with a plant with purple flowers and green, wrinkled seeds (heterozygous for flower color but homozygous recessive for the other two characters). Using Mendelian symbols, our cross is $PpYyRr \times Ppyyrr$. What fraction of offspring from this cross are predicted to exhibit the recessive phenotypes for *at least two* of the three characters?

To answer this question, we can start by listing all genotypes we could get that fulfill this condition: $ppyyRr$, $ppYyrr$, $Ppyyrr$, $PPyyrr$, and $ppyyrr$. (Because the condition is *at least two* recessive traits, it includes the last genotype, which shows all three recessive traits.) Next, we calculate the probability for each of these genotypes resulting from our $PpYyRr \times Ppyyrr$ cross by multiplying together the individual probabilities for the allele pairs, just as we did in our dihybrid example. Note that in a cross involving heterozygous and homozygous allele pairs (for example, $Yy \times yy$), the probability of heterozygous offspring is $\frac{1}{2}$ and the probability of homozygous offspring is $\frac{1}{2}$. Finally, we use the addition rule to add the probabilities for all the different genotypes that fulfill the condition of at least two recessive traits resulting from our $PpYyRr \times Ppyyrr$ cross, as shown below:

$ppyyRr$	$\frac{1}{4}$ (probability of pp) $\times \frac{1}{2}$ (yy) $\times \frac{1}{2}$ (Rr)	$= \frac{1}{16}$
$ppYyrr$	$\frac{1}{4} \times \frac{1}{2} \times \frac{1}{2}$	$= \frac{1}{16}$
$Ppyyrr$	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	$= \frac{2}{16}$
$PPyyrr$	$\frac{1}{4} \times \frac{1}{2} \times \frac{1}{2}$	$= \frac{1}{16}$
$ppyyrr$	$\frac{1}{4} \times \frac{1}{2} \times \frac{1}{2}$	$= \frac{1}{16}$
Chance of <i>at least two</i> recessive traits		$= \frac{6}{16}$ or $\frac{3}{8}$

In time, you'll be able to solve genetics problems faster by using the rules of probability than by filling in Punnett squares.

We cannot predict with certainty the exact numbers of progeny of different genotypes resulting from a genetic cross. But the rules of probability give us the *likelihood* of various outcomes. Usually, the larger the sample size, the closer the results will conform to our predictions. Mendel understood

this statistical feature of inheritance and had a keen sense of the rules of chance. It was for this reason that he set up his experiments so as to generate, and then count, large numbers of offspring from his crosses.

CONCEPT CHECK 14.2

1. An individual with cystic fibrosis (genotype ff) marries a heterozygous individual who carries a copy of the recessive allele (genotype Ff). What proportions of the offspring are expected to be homozygous dominant, homozygous recessive, and heterozygous?
2. If two organisms, both with the genotype $AaBb$, are mated, what is the probability of obtaining the genotypes $AABB$ and $AaBb$ in the F_2 generation?
3. **WHAT IF? >** Three characters (flower color, seed color, and pod shape) are considered in a cross between two pea plants: $PpYyli \times ppYyii$. What fraction of offspring is predicted to be homozygous recessive for at least two of the three characters?

For suggested answers, see Appendix A.

CONCEPT 14.3

Inheritance patterns are often more complex than predicted by simple Mendelian genetics

In the 20th century, geneticists extended Mendelian principles not only to diverse organisms, but also to patterns of inheritance more complex than those described by Mendel. For the work that led to his two laws of inheritance, Mendel chose pea plant characters that turn out to have a relatively simple genetic basis: Each character is determined by one gene, for which there are only two alleles, one completely dominant and the other completely recessive. (There is one exception: Mendel's pod shape character is actually determined by two genes.) Not all heritable characters are determined so simply, and the relationship between genotype and phenotype is rarely so straightforward. Mendel himself realized that he could not explain the more complicated patterns he observed in crosses involving other pea characters or other plant species. This does not diminish the utility of Mendelian genetics, however, because the basic principles of segregation and independent assortment apply even to more complex patterns of inheritance. In this section, we will extend Mendelian genetics to hereditary patterns that were not reported by Mendel.

Extending Mendelian Genetics for a Single Gene

The inheritance of characters determined by a single gene deviates from simple Mendelian patterns when alleles are not completely dominant or recessive, when a particular gene has

more than two alleles, or when a single gene produces multiple phenotypes. We will describe examples of each of these situations in this section.

Degrees of Dominance

Alleles can show different degrees of dominance and recessiveness in relation to each other. In Mendel's classic pea crosses, the F_1 offspring always looked like one of the two parental varieties because one allele in a pair showed **complete dominance** over the other. In such situations, the phenotypes of the heterozygote and the dominant homozygote are indistinguishable (see Figure 14.6).

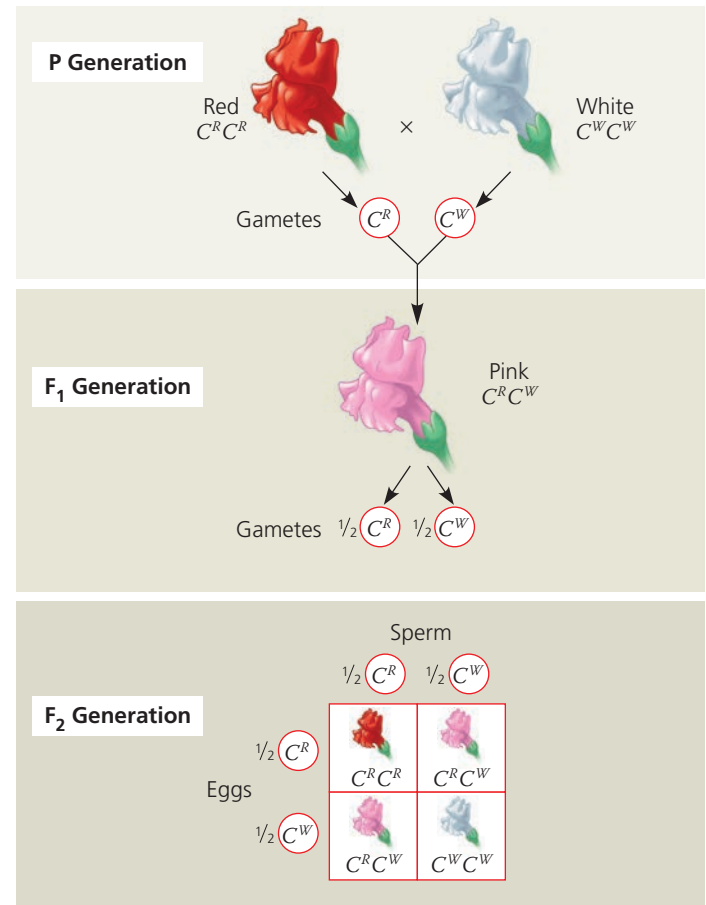
For some genes, however, neither allele is completely dominant, and the F_1 hybrids have a phenotype somewhere between those of the two parental varieties. This phenomenon, called **incomplete dominance**, is seen when red snapdragons are crossed with white snapdragons: All the F_1 hybrids have pink flowers (Figure 14.10). This third, intermediate phenotype results from flowers of the heterozygotes having less red pigment than the red homozygotes. (This is unlike the case of Mendel's pea plants, where the Pp heterozygotes make enough pigment for the flowers to be purple, indistinguishable from those of PP plants.)

At first glance, incomplete dominance of either allele seems to provide evidence for the blending hypothesis of inheritance, which would predict that the red or white trait could never reappear among offspring of the pink hybrids. In fact, interbreeding F_1 hybrids produces F_2 offspring with a phenotypic ratio of one red to two pink to one white. (Because heterozygotes have a separate phenotype, the genotypic and phenotypic ratios for the F_2 generation are the same, 1:2:1.) The segregation of the red-flower and white-flower alleles in the gametes produced by the pink-flowered plants confirms that the alleles for flower color are heritable factors that maintain their identity in the hybrids; that is, inheritance is particulate.

Another variation on dominance relationships between alleles is called **codominance**; in this variation, the two alleles each affect the phenotype in separate, distinguishable ways. For example, the human MN blood group is determined by codominant alleles for two specific molecules located on the surface of red blood cells, the M and N molecules. A single gene locus, at which two allelic variations are possible, determines the phenotype of this blood group. Individuals homozygous for the M allele (MM) have red blood cells with only M molecules; individuals homozygous for the N allele (NN) have red blood cells with only N molecules. But *both* M and N molecules are present on the red blood cells of individuals heterozygous for the M and N alleles (MN). Note that the MN phenotype is *not* intermediate between the M and N phenotypes, which distinguishes codominance from incomplete dominance. Rather, *both* M and N phenotypes are exhibited by heterozygotes, since both molecules are present.

Figure 14.10 Incomplete dominance in snapdragon color.

When red snapdragons are crossed with white ones, the F_1 hybrids have pink flowers. Segregation of alleles into gametes of the F_1 plants results in an F_2 generation with a 1:2:1 ratio for both genotype and phenotype. Neither allele is dominant, so rather than using upper- and lowercase letters, we use the letter C with a superscript to indicate an allele for flower color: C^R for red and C^W for white.



? Suppose a classmate argues that this figure supports the blending hypothesis for inheritance. What might your classmate say, and how would you respond?

Animation: Incomplete Dominance in "MendAliens"

The Relationship Between Dominance and Phenotype

We've now seen that the relative effects of two alleles range from complete dominance of one allele, to incomplete dominance of either allele, to codominance of both alleles. It is important to understand that an allele is called *dominant* because it is seen in the phenotype, not because it somehow subdues a recessive allele. Alleles are simply variations in a gene's nucleotide sequence (see Figure 14.4). When a dominant allele coexists with a recessive allele in a heterozygote, they do not actually interact at all. It is in the pathway from genotype to phenotype that dominance and recessiveness come into play.

To illustrate the relationship between dominance and phenotype, we can use one of the characters Mendel

studied—round versus wrinkled pea seed shape. The dominant allele (round) codes for an enzyme that helps convert an unbranched form of starch to a branched form in the seed. The recessive allele (wrinkled) codes for a defective form of this enzyme, leading to an accumulation of unbranched starch, which causes excess water to enter the seed by osmosis. Later, when the seed dries, it wrinkles. If a dominant allele is present, no excess water enters the seed and it does not wrinkle when it dries. One dominant allele results in enough of the enzyme to synthesize adequate amounts of branched starch, which means that dominant homozygotes and heterozygotes have the same phenotype: round seeds.

A closer look at the relationship between dominance and phenotype reveals an intriguing fact: For any character, the observed dominant/recessive relationship of alleles depends on the level at which we examine phenotype. **Tay-Sachs disease**, an inherited disorder in humans, is an example. The brain cells of a child with Tay-Sachs disease cannot metabolize certain lipids because a crucial enzyme does not work properly. As these lipids accumulate in brain cells, the child begins to suffer seizures, blindness, and degeneration of motor and mental performance and dies within a few years.

Only children who inherit two copies of the Tay-Sachs allele (homozygotes) have the disease. Thus, at the *organismal* level, the Tay-Sachs allele qualifies as recessive. However, the activity level of the lipid-metabolizing enzyme in heterozygotes is intermediate between the activity level in individuals homozygous for the normal allele and the activity level in individuals with Tay-Sachs disease. (The term normal is used in the genetic sense to refer to the allele coding for the enzyme that functions properly.) The intermediate phenotype observed at the *biochemical* level is characteristic of incomplete dominance of either allele. Fortunately, the heterozygote condition does not lead to disease symptoms, apparently because half the normal enzyme activity is sufficient to prevent lipid accumulation in the brain. Extending our analysis to yet another level, we find that heterozygous individuals produce equal numbers of normal and dysfunctional enzyme molecules. Thus, at the *molecular* level, the normal allele and the Tay-Sachs allele are codominant. As you can see, whether alleles appear to be completely dominant, incompletely dominant, or codominant depends on the level at which the phenotype is analyzed.

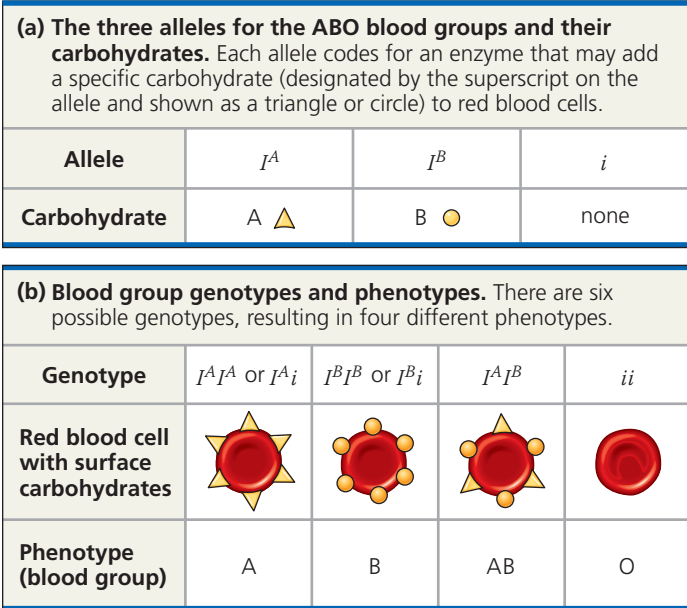
Frequency of Dominant Alleles Although you might assume that the dominant allele for a particular character would be more common than the recessive allele, this is not always the case. For an example of a rare dominant allele, about one baby out of 400 in the United States is born with extra fingers or toes, a condition known as polydactyly. Some cases are caused by the presence of a dominant allele. The low frequency of polydactyly indicates that the recessive allele, which results in five digits per appendage, is far more prevalent than the dominant allele in the population.

In Concept 23.3, you will learn how relative frequencies of alleles in a population are affected by natural selection.

Multiple Alleles

Only two alleles exist for the pea characters that Mendel studied, but most genes exist in more than two allelic forms. The ABO blood groups in humans, for instance, are determined by that person’s two alleles of the blood group gene; there are three possible alleles: I^A , I^B , and i . A person’s blood group may be one of four types: A, B, AB, or O. These letters refer to two carbohydrates—A and B—that may be found attached to specific cell-surface molecules on red blood cells. An individual’s blood cells may have carbohydrate A (type A blood), carbohydrate B (type B), both (type AB), or neither (type O), as shown in **Figure 14.11**. Matching compatible blood groups is critical for safe blood transfusions (see Concept 47.3).

Figure 14.11 Multiple alleles for the ABO blood groups. The four blood groups result from different combinations of three alleles.



VISUAL SKILLS Based on the surface carbohydrate phenotype in (b), what are the dominance relationships among the alleles?

Pleiotropy

So far, we have treated Mendelian inheritance as though each gene affects only one phenotypic character. Most genes, however, have multiple phenotypic effects, a property called **pleiotropy** (from the Greek *pleion*, more). In humans, for example, pleiotropic alleles are responsible for the multiple symptoms associated with certain hereditary diseases, such as cystic fibrosis and sickle-cell disease, discussed later in this chapter. In the garden pea, the gene that determines flower color also affects the color of the coating on the outer surface of the seed, which can be gray or white. Given the intricate

molecular and cellular interactions responsible for an organism's development and physiology, it isn't surprising that a single gene can affect a number of characters.

Extending Mendelian Genetics for Two or More Genes

Dominance relationships, multiple alleles, and pleiotropy all have to do with the effects of the alleles of a single gene. We now consider two situations in which two or more genes are involved in determining a particular phenotype. In the first case, called epistasis, one gene affects the phenotype of another because the two gene products interact; in the second case, called polygenic inheritance, multiple genes independently affect a single trait.

Epistasis

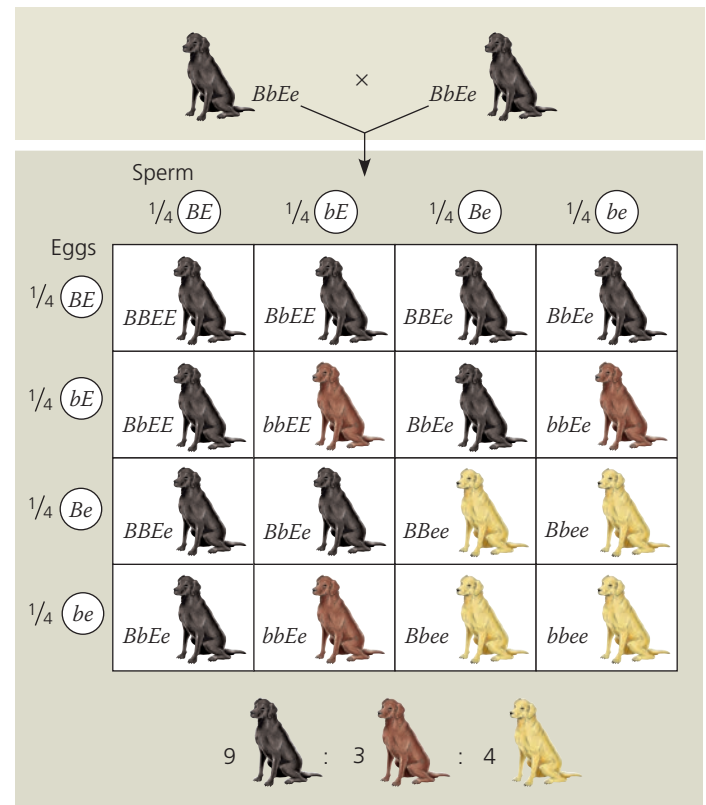
In **epistasis** (from the Greek for “standing upon”), the phenotypic expression of a gene at one locus alters that of a gene at a second locus. An example will help clarify this concept. In Labrador retrievers (commonly called “Labs”), black coat color is dominant to brown. Let's designate *B* and *b* as the two alleles for this character. For a Lab to have brown fur, its genotype must be *bb*; these dogs are called chocolate Labs. But there is more to the story. A second gene determines whether or not pigment will be deposited in the hair. The dominant allele, symbolized by *E*, results in the deposition of either black or brown pigment, depending on the genotype at the first locus. But if the Lab is homozygous recessive for the second locus (*ee*), then the coat is yellow, regardless of the genotype at the black/brown locus (yellow Labs). In this case, the gene for pigment deposition (*E/e*) is said to be epistatic to the gene that codes for black or brown pigment (*B/b*).

What happens if we mate black Labs that are heterozygous for both genes (*BbEe*)? Although the two genes affect the same phenotypic character (coat color), they follow the law of independent assortment. Thus, our breeding experiment represents an *F*₁ dihybrid cross, like those that produced a 9:3:3:1 ratio in Mendel's experiments. We can use a Punnett square to represent the genotypes of the *F*₂ offspring (Figure 14.12). As a result of epistasis, the phenotypic ratio among the *F*₂ offspring is 9 black to 3 chocolate to 4 yellow Labs. Other types of epistatic interactions produce different ratios, but all are modified versions of 9:3:3:1.

Polygenic Inheritance

Mendel studied characters that could be classified on an either-or basis, such as purple versus white flower color. But many characters, such as human skin color and height, are not one of two discrete characters, but instead vary in the population in gradations along a continuum. These are called **quantitative characters**. Quantitative variation usually indicates **polygenic inheritance**, an additive effect of two

▼ **Figure 14.12 An example of epistasis.** This Punnett square illustrates the genotypes and phenotypes predicted for offspring of matings between two black Labrador retrievers of genotype *BbEe*. The *E/e* gene, which is epistatic to the *B/b* gene coding for hair pigment, controls whether or not pigment of any color will be deposited in the hair.

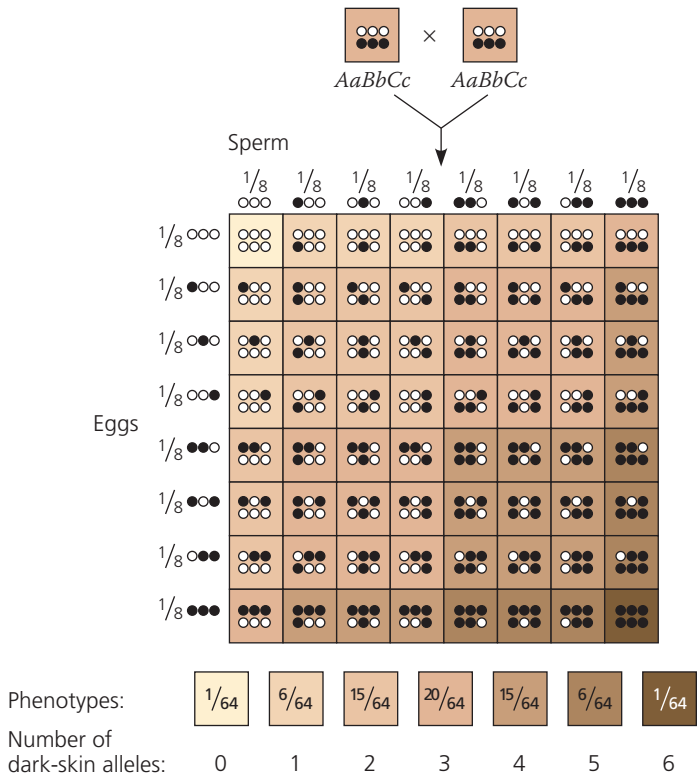


VISUAL SKILLS ► Compare the four squares in the lower right of this Punnett square with those in Figure 14.8. Explain the genetic basis for the difference between the ratio (9:3:4) of phenotypes seen in this cross and the 9:3:3:1 ratio seen in Figure 14.8.

or more genes on a single phenotypic character. (In a way, this is the converse of pleiotropy, where a single gene affects several phenotypic characters.) Height is a good example of polygenic inheritance: In 2014, a genomic study of over 250,000 people found almost 700 genetic variations associated with over 180 genes that affect height. Many variations were in or near genes involved in biochemical pathways affecting growth of the skeleton, but others were associated with genes not obviously related to growth.

Skin pigmentation in humans is also controlled by many separately inherited genes. Here, we'll simplify the story in order to understand the concept of polygenic inheritance. Let's consider three genes, with a dark-skin allele for each gene (*A*, *B*, or *C*) contributing one “unit” of darkness (also a simplification) to the phenotype and being incompletely dominant to the other allele (*a*, *b*, or *c*). In our model, an *AABBCC* person would be very dark, whereas an *aabbcc* individual would be very light. An *AaBbCc* person would have skin of an intermediate shade. Because the alleles have a cumulative effect, the genotypes *AaBbCc* and *AABbcc* would make the same genetic contribution (three units) to skin

▼ **Figure 14.13 A simplified model for polygenic inheritance of skin color.** In this model, three separately inherited genes affect skin color. The heterozygous individuals ($AaBbCc$) represented by the two rectangles at the top of this figure each carry three dark-skin alleles (black circles, which represent A , B , or C) and three light-skin alleles (white circles, which represent a , b , or c). The Punnett square shows all the possible genetic combinations in gametes and offspring of many hypothetical matings between these heterozygotes. The results are summarized by the phenotypic frequencies (fractions) under the Punnett square. (The phenotypic ratio of the skin colors shown in the boxes is 1:6:15:20:15:6:1.)



darkness. There are seven skin color phenotypes that could result from a mating between $AaBbCc$ heterozygotes, as shown in **Figure 14.13**. In a large number of such matings, the majority of offspring would be expected to have intermediate phenotypes (skin color in the middle range). You can graph the predictions from the Punnett square in the **Scientific Skills Exercise**. Environmental factors, such as exposure to the sun, also affect the skin color phenotype.

Nature and Nurture: The Environmental Impact on Phenotype

Another departure from simple Mendelian genetics arises when the phenotype for a character depends on environment as well as genotype. A single tree, locked into its inherited genotype, has leaves that vary in size, shape, and greenness, depending on their exposure to wind and sun. For humans, nutrition influences height, exercise alters build, sun-tanning darkens the skin, and experience improves performance on intelligence tests. Even identical twins, who are genetic

equals, accumulate phenotypic differences as a result of their unique experiences.

Whether human characters are more influenced by genes or the environment—in everyday terms, nature versus nurture—is a debate that we will not attempt to settle here. We can say, however, that a genotype generally is not associated with a rigidly defined phenotype, but rather with a range of phenotypic possibilities due to environmental influences (**Figure 14.14**). For some characters, such as the ABO blood group system, the phenotypic range has no breadth whatsoever; that is, a given genotype mandates a very specific phenotype. Other characters, such as a person's blood count of red and white cells, vary quite a bit, depending on such factors as the altitude, the customary level of physical activity, and the presence of infectious agents.

▼ Figure 14.14 The effect of environment on phenotype.

The outcome of a genotype lies within a phenotypic range that depends on the environment in which the genotype is expressed. For example, the acidity and free aluminum content of the soil affect the color of hydrangea flowers, which range from pink (basic soil) to blue-violet (acidic soil). Free aluminum is necessary for bluer colors.



(a) Hydrangeas grown in basic soil



(b) Hydrangeas of the same genetic variety grown in acidic soil with free aluminum

Generally, the phenotypic range is broadest for polygenic characters. Environment contributes to the quantitative nature of these characters, as we have seen in the continuous variation of skin color. Geneticists refer to such characters as **multifactorial**, meaning that many factors, both genetic and environmental, collectively influence phenotype.

A Mendelian View of Heredity and Variation

We have now broadened our view of Mendelian inheritance by exploring degrees of dominance as well as multiple alleles, pleiotropy, epistasis, polygenic inheritance, and the phenotypic impact of the environment. How can we integrate these refinements into a comprehensive theory of Mendelian genetics? The key is to make the transition from the reductionist emphasis on single genes and phenotypic characters to the emergent properties of the organism as a whole, one of the themes of this book.

The term *phenotype* can refer not only to specific characters, such as flower color and blood group, but also to an organism in its entirety—all aspects of its physical

SCIENTIFIC SKILLS EXERCISE

Making a Histogram and Analyzing a Distribution Pattern

What Is the Distribution of Phenotypes Among Offspring of Two Parents Who Are Both Heterozygous for Three Additive Genes? Human skin color is a polygenic trait that is determined by the additive effects of many different genes. In this exercise, you will work with a simplified model of skin-color genetics where only three genes are assumed to affect the darkness of skin color and where each gene has two alleles—dark or light (see Figure 14.13). In this model, each dark allele contributes equally to the darkness of skin color, and each pair of alleles segregates independently of any other pair. Using a type of graph called a histogram, you will determine the distribution of phenotypes of offspring with different numbers of dark-skin alleles. (For additional information about graphs, see the Scientific Skills Review in Appendix F.)

How This Model Is Analyzed To predict the phenotypes of the offspring of parents heterozygous for the three genes in our simplified model, we can use the Punnett square in Figure 14.13. The heterozygous individuals ($AaBbCc$) represented by the two rectangles at the top of that figure each carry three dark-skin alleles (black circles, which represent A , B , or C) and three light-skin alleles (white circles, which represent a , b , or c). The Punnett square shows all the possible genetic combinations in gametes and in offspring of a large number of hypothetical matings between these heterozygotes.

Predictions from the Punnett Square If we assume that each square in the Punnett square represents one offspring of the heterozygous $AaBbCc$ parents, then the squares below show the possible skin-color phenotypes and their predicted frequencies. Below the squares is the number of dark-skin alleles for each phenotype.

Phenotypes:							
Number of dark-skin alleles:	0	1	2	3	4	5	6

appearance, internal anatomy, physiology, and behavior. Similarly, the term *genotype* can refer to an organism's entire genetic makeup, not just its alleles for a single genetic locus. In most cases, a gene's impact on phenotype is affected by other genes and by the environment. In this integrated view of heredity and variation, an organism's phenotype reflects its overall genotype and unique environmental history.

Considering all that can occur in the pathway from genotype to phenotype, it is indeed impressive that Mendel could uncover the fundamental principles governing the transmission of individual genes from parents to offspring. Mendel's laws of segregation and of independent assortment explain heritable variations in terms of alternative forms of genes (hereditary "particles," now known as the alleles of genes) that are passed along, generation after generation, according to simple rules of probability. This theory of inheritance is equally valid for peas, flies, fishes, birds, and human beings—indeed, for any organism with a sexual life cycle. Furthermore, by extending the principles of segregation and independent assortment to help explain such hereditary



INTERPRET THE DATA

1. A histogram is a bar graph that shows the distribution of numeric data (here, the number of dark-skin alleles). To make a histogram of the allele distribution, put skin color (as the number of dark-skin alleles) along the x-axis and predicted number of offspring (out of 64) with each phenotype on the y-axis. There are no gaps in these allele data, so draw the bars next to each other with no space in between.
2. You can see that the skin-color phenotypes are not distributed uniformly. (a) Which phenotype has the highest frequency? Draw a vertical dashed line through that bar. (b) Distributions of values like this one tend to show one of several common patterns. Sketch a rough curve that approximates the values and look at its shape. Is it symmetrically distributed around a central peak value (a "normal distribution," sometimes called a bell curve); is it skewed to one end of the x-axis or the other (a "skewed distribution"); or does it show two apparent groups of frequencies (a "bimodal distribution")? Explain the reason for the curve's shape. (It will help to read the text description that supports Figure 14.13.)



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Further Reading R. A. Sturm, A golden age of human pigmentation genetics, *Trends in Genetics* 22:464–468 (2006).

patterns as epistasis and quantitative characters, we begin to see how broadly Mendelian genetics applies. From Mendel's abbey garden came a theory of particulate inheritance that anchors modern genetics. In the last section of this chapter, we will apply Mendelian genetics to human inheritance, with emphasis on the transmission of hereditary diseases.

CONCEPT CHECK 14.3

1. *Incomplete dominance* and *epistasis* are both terms that define genetic relationships. What is the most basic distinction between these terms?
2. If a man with type AB blood marries a woman with type O, what blood types would you expect in their children? What fraction would you expect of each type?
3. **WHAT IF? >** A rooster with gray feathers and a hen of the same phenotype produce 15 gray, 6 black, and 8 white chicks. What is the simplest explanation for the inheritance of these colors in chickens? What phenotypes would you expect in the offspring of a cross between a gray rooster and a black hen?

For suggested answers, see Appendix A.

CONCEPT 14.4

Many human traits follow Mendelian patterns of inheritance

Peas are convenient subjects for genetic research, but humans are not. The human generation span is long—about 20 years—and human parents produce many fewer offspring than peas and most other species. Even more important, it wouldn't be ethical to ask pairs of humans to breed so that the phenotypes of their offspring could be analyzed! In spite of these constraints, the study of human genetics continues, spurred on by our desire to understand our own inheritance and to develop treatments and cures for human genetically based diseases. New molecular biological techniques have led to many breakthrough discoveries, as we will see in Concept 19.4, but basic Mendelian genetics endures as the foundation of human genetics.

Pedigree Analysis

Unable to manipulate the mating patterns of people, geneticists instead analyze the results of matings that have already occurred. They do so by collecting information about a family's history for a particular trait and assembling this information into a family tree describing the traits of parents and children across the generations—a family **pedigree**.

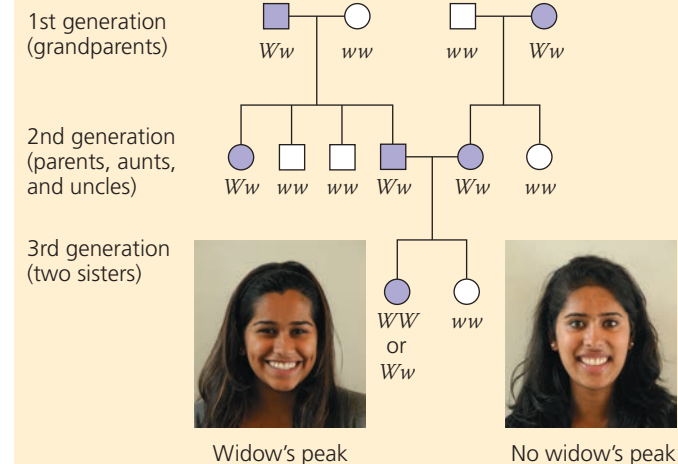
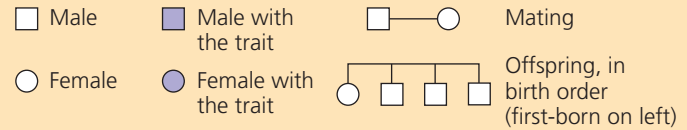
Figure 14.15a shows a three-generation pedigree that traces the occurrence of a pointed contour of the hairline on the forehead. This trait, called a widow's peak, is due to a dominant allele, W . Because the widow's-peak allele is dominant, all individuals who lack a widow's peak must be homozygous recessive (ww). The two grandparents with widow's peaks must have the Ww genotype, since some of their offspring are homozygous recessive. The offspring in the second generation who *do* have widow's peaks must also be heterozygous, because they are the products of $Ww \times ww$ matings. The third generation in this pedigree consists of two sisters. The one who has a widow's peak could be either homozygous (WW) or heterozygous (Ww), given what we know about the genotypes of her parents (both Ww).

Figure 14.15b is a pedigree of the same family, but this time we focus on a recessive trait, the inability of individuals to taste a chemical called PTC (phenylthiocarbamide). Compounds similar to PTC are found in broccoli, brussels sprouts, and related vegetables and account for the bitter taste some people report when eating these foods. We'll use t for the recessive allele and T for the dominant allele, which results in the ability to taste PTC. As you work your way through the pedigree, notice once again that you can apply what you have learned about Mendelian inheritance to understand the genotypes shown for the family members.

An important application of a pedigree is to help us calculate the probability that a future child will have a particular

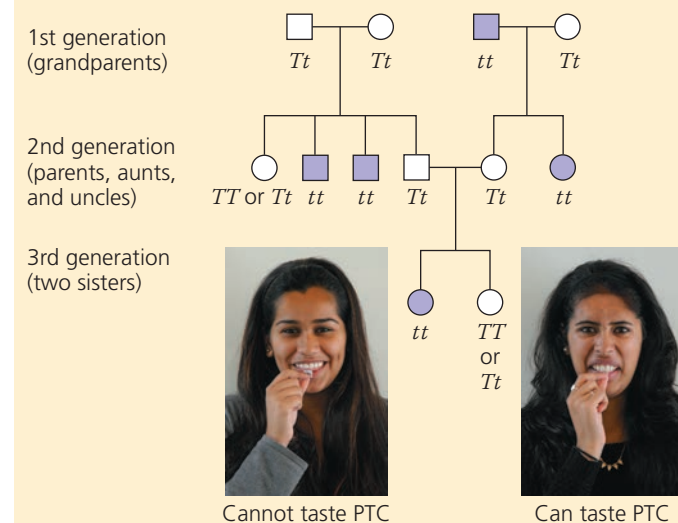
Figure 14.15 Pedigree analysis. Each of these pedigrees traces a trait through three generations of the same family. The two traits have different inheritance patterns, as shown by the pedigrees. (Note: While most traits are not determined by a single gene, that is generally agreed to be the case for these two traits.)

Key



(a) Is a widow's peak a dominant or recessive trait?

Tips for pedigree analysis: Notice in the third generation that the second-born daughter lacks a widow's peak, although both of her parents had the trait. Such a pattern indicates that the trait is due to a dominant allele. If it were due to a recessive allele, and both parents had the recessive phenotype (straight hairline), *all* of their offspring would also have the recessive phenotype.



(b) Is the inability to taste a chemical called PTC a dominant or recessive trait?

Tips for pedigree analysis: Notice that the first-born daughter in the third generation has the trait (is unable to taste PTC), although both parents lack that trait (they *can* taste PTC). Such a pattern is explained if the non-taster phenotype is due to a recessive allele. (If it were due to a dominant allele, then at least one parent would also have had the trait.)

genotype and phenotype. Suppose that the couple represented in the second generation of Figure 14.15 decides to have one more child. What is the probability that the child will have a widow's peak? This is equivalent to a Mendelian F_1 monohybrid cross ($Ww \times Ww$), and therefore the probability that a child will inherit a dominant allele and have a widow's peak is $\frac{3}{4}$ ($\frac{1}{4} WW + \frac{1}{2} Ww$). What is the probability that the child will be unable to taste PTC? We can also treat this as a monohybrid cross ($Tt \times Tt$), but this time we want to know the chance that the offspring will be homozygous recessive (tt). That probability is $\frac{1}{4}$. Finally, what is the chance that the child will have a widow's peak *and* be unable to taste PTC? Assuming that the genes for these two characters are on different chromosomes, the two pairs of alleles will assort independently in this dihybrid cross ($WwTt \times WwTt$). Therefore, we can use the multiplication rule: $\frac{3}{4}$ (chance of widow's peak) $\times \frac{1}{4}$ (chance of inability to taste PTC) = $\frac{3}{16}$ (chance of widow's peak and inability to taste PTC).

Pedigrees are a more serious matter when the alleles in question cause disabling or deadly diseases instead of innocuous human variations such as hairline or inability to taste an innocuous chemical. However, for disorders inherited as simple Mendelian traits, the same techniques of pedigree analysis apply.

Recessively Inherited Disorders

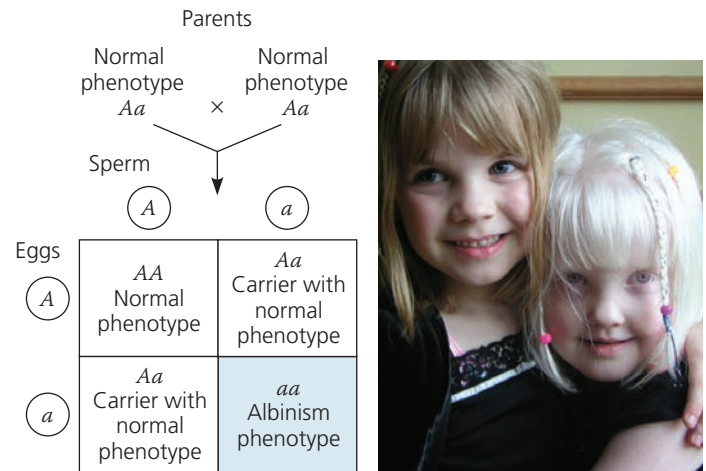
Thousands of genetic disorders are known to be inherited as simple recessive traits. These disorders range in severity from relatively mild, such as albinism (lack of pigmentation, which results in susceptibility to skin cancers and vision problems), to life-threatening, such as cystic fibrosis.

The Behavior of Recessive Alleles

How can we account for the behavior of alleles that cause recessively inherited disorders? Recall that genes code for proteins of specific function. An allele that causes a genetic disorder (let's call it allele a) codes for either a malfunctioning protein or no protein at all. In the case of disorders classified as recessive, heterozygotes (Aa) typically have the normal phenotype because one copy of the normal allele (A) produces a sufficient amount of the specific protein. Thus, a recessively inherited disorder shows up only in the homozygous individuals (aa) who inherit a recessive allele from each parent. Although phenotypically normal with regard to the disorder, heterozygotes may transmit the recessive allele to their offspring and thus are called **carriers**. **Figure 14.16** illustrates these ideas using albinism as an example.

Most people who have recessive disorders are born to parents who are carriers of the disorder but have a normal phenotype, as is the case shown in the Punnett square in Figure 14.16. A mating between two carriers corresponds to a Mendelian F_1 monohybrid cross, so the predicted genotypic ratio for the offspring is 1 AA : 2 Aa : 1 aa . Thus, each child has

✓ Figure 14.16 Albinism: a recessive trait. One of the two sisters shown here has normal coloration; the other has albinism. Most recessive homozygotes are born to parents who are carriers of the disorder but themselves have a normal phenotype, the case shown in the Punnett square.



? What is the probability that the sister with normal coloration is a carrier of the albinism allele?

a $\frac{1}{4}$ chance of inheriting a double dose of the recessive allele; in the case of albinism, such a child will have albinism. From the genotypic ratio, we also can see that out of three offspring with the *normal* phenotype (one AA plus two Aa), two are predicted to be heterozygous carriers, a $\frac{2}{3}$ chance. Recessive homozygotes could also result from $Aa \times aa$ and $aa \times Aa$ matings, but if the disorder is lethal before reproductive age or results in sterility (neither of which is true for albinism), no aa individuals will reproduce. Even if recessive homozygotes are able to reproduce, such matings will occur relatively rarely because these individuals account for a much smaller percentage of the population than heterozygous carriers (for reasons we'll examine in Concept 23.2).

In general, genetic disorders are not evenly distributed among all groups of people. For example, the incidence of Tay-Sachs disease, which we described earlier in this chapter, is disproportionately high among Ashkenazic Jews, Jewish people whose ancestors lived in central Europe. In that population, Tay-Sachs disease occurs in one out of 3,600 births, an incidence about 100 times greater than that among non-Jews or Mediterranean (Sephardic) Jews. This uneven distribution results from the different genetic histories of the world's peoples during less technological times, when populations were more geographically (and hence genetically) isolated.

When a disease-causing recessive allele is rare, it is relatively unlikely that two carriers of the same harmful allele will meet and mate. The probability of passing on recessive traits increases greatly, however, if the man and woman are close relatives (for example, siblings or first cousins). This is because people with recent common ancestors are more likely to carry the same recessive alleles than are unrelated people.

Thus, these consanguineous (“same blood”) matings, indicated in pedigrees by double lines, are more likely to produce offspring homozygous for recessive traits—including harmful ones. Such effects can be observed in many types of domesticated and zoo animals that have become inbred.

Although it is generally agreed that inbreeding causes an increase in autosomal recessive conditions compared to those resulting from matings between unrelated parents, there is debate among geneticists about exactly how much human consanguinity increases the risk of inherited diseases. For one thing, many harmful alleles have such severe effects that a homozygous embryo spontaneously aborts long before birth. Most societies and cultures have laws or taboos forbidding marriages between close relatives. These rules may have evolved out of empirical observation that in most populations, stillbirths and birth defects are more common when parents are closely related. Social and economic factors have also influenced the development of customs and laws against consanguineous marriages.

Cystic Fibrosis

The most common lethal genetic disease in the United States is **cystic fibrosis**, which strikes one out of every 2,500 people of European descent but is much rarer in other groups. Among people of European descent, one out of 25 (4%) are carriers of the cystic fibrosis allele. The normal allele for this gene codes for a membrane protein that functions in the transport of chloride ions between certain cells and the extracellular fluid. These chloride transport channels are defective or absent in the plasma membranes of children who inherit two recessive alleles for cystic fibrosis. The result is an abnormally high concentration of intracellular chloride, which causes an uptake of water due to osmosis. This in turn causes the mucus that coats certain cells to become thicker and stickier than normal. The mucus builds up in the pancreas, lungs, digestive tract, and other organs, leading to multiple (pleiotropic) effects, including poor absorption of nutrients from the intestines, chronic bronchitis, and recurrent bacterial infections.

Untreated, cystic fibrosis can cause death by the age of 5. Daily doses of antibiotics to stop infection, gentle pounding on the chest to clear mucus from clogged airways, and other therapies can prolong life. In the United States, more than half of those with cystic fibrosis now survive into their 30s and beyond.

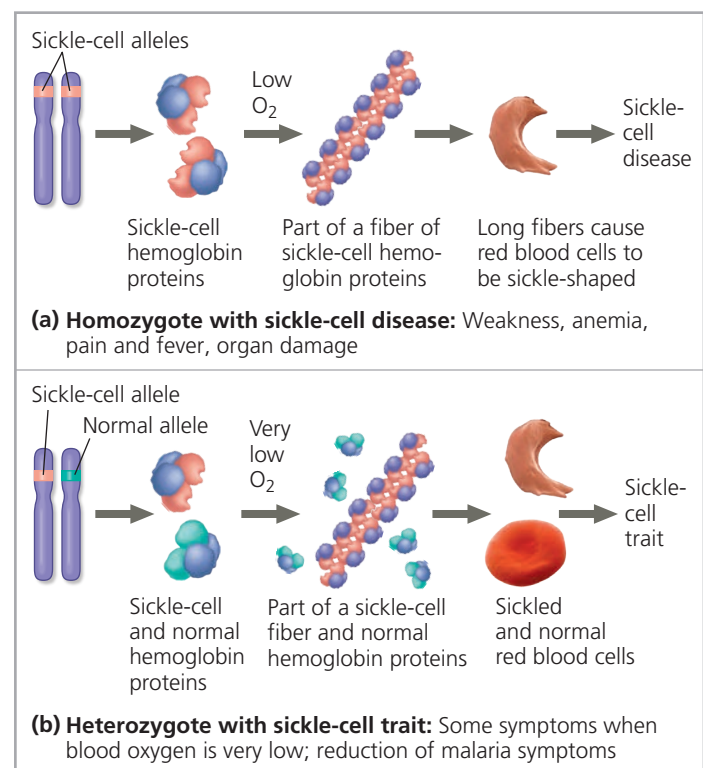
Sickle-Cell Disease: A Genetic Disorder with Evolutionary Implications

EVOLUTION The most common inherited disorder among people of African descent is **sickle-cell disease**, which affects one out of 400 African-Americans. Sickle-cell disease is caused by the substitution of a single amino acid in the hemoglobin protein of red blood cells; in homozygous individuals, all

hemoglobin is of the sickle-cell (abnormal) variety. When the oxygen content of an affected individual’s blood is low (at high altitudes or under physical stress, for instance), the sickle-cell hemoglobin proteins aggregate into long fibers that deform the red cells into a sickle shape (see Figure 5.19). Sickled cells may clump and clog small blood vessels, often leading to other symptoms throughout the body, including physical weakness, pain, organ damage, and even stroke and paralysis. Regular blood transfusions can ward off brain damage in children with sickle-cell disease, and new drugs can help prevent or treat other problems. There is currently no widely available cure, but the disease is the target of ongoing gene therapy research.

Although two sickle-cell alleles are necessary for an individual to manifest full-blown sickle-cell disease and thus the condition is considered a recessive one, the presence of one sickle-cell allele can affect the phenotype. Thus, at the organismal level, the normal allele is incompletely dominant to the sickle-cell allele (**Figure 14.17**). At the molecular level, the two alleles are codominant; both normal and abnormal (sickle-cell) hemoglobins are made in heterozygotes (carriers), who are said to have *sickle-cell trait*. (Here the word “trait” is used to distinguish this condition from full-blown sickle-cell disease, thus it is used differently from its definition earlier in the chapter—any variant of a phenotypic character.) Heterozygotes are usually healthy but may suffer some symptoms during long periods of reduced blood oxygen.

▼ **Figure 14.17 Sickle-cell disease and sickle-cell trait.**



HHMI Animation:
Sickle-Cell Disease



About one out of ten African-Americans have sickle-cell trait, an unusually high frequency of heterozygotes for an allele with severe detrimental effects in homozygotes. Why haven't evolutionary processes resulted in the disappearance of the allele among this population? One explanation is that having a single copy of the sickle-cell allele reduces the frequency and severity of malaria attacks, especially among young children. The malaria parasite spends part of its life cycle in red blood cells (see Figure 28.16), and the presence of even heterozygous amounts of sickle-cell hemoglobin results in lower parasite densities and hence reduced malaria symptoms. Thus, in tropical Africa, where infection with the malaria parasite is common, the sickle-cell allele confers an advantage to heterozygotes even though it is harmful in the homozygous state. (The balance between these two effects will be discussed in Concept 23.4; see Make Connections Figure 23.18.) The relatively high frequency of African-Americans with sickle-cell trait is a vestige of their African ancestry.



HHMI Video: The Making of the Fittest: Natural Selection in Humans (Sickle-Cell Disease)

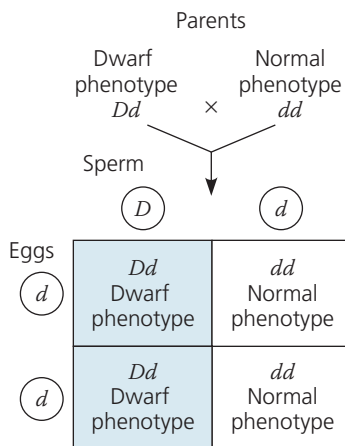


Dominantly Inherited Disorders

Although many harmful alleles are recessive, a number of human disorders are due to dominant alleles. One example is *achondroplasia*, a form of dwarfism that occurs in one of every 25,000 people. Heterozygous individuals have the dwarf phenotype (Figure 14.18). Therefore, all people who do not have achondroplasia—99.99% of the population—are homozygous for the recessive allele. Like the presence of extra fingers or toes mentioned earlier, achondroplasia is a trait for which the recessive allele is much more prevalent than the corresponding dominant allele.

▼ Figure 14.18 Achondroplasia: a dominant trait.

Dr. Michael C. Ain has achondroplasia, a form of dwarfism caused by a dominant allele. This has inspired his work: He is a specialist in the repair of bone defects caused by achondroplasia and other disorders. The dominant allele (*D*) might have arisen as a mutation in the egg or sperm of a parent or could have been inherited from an affected parent, as shown for an affected father in the Punnett square.



Unlike achondroplasia, which is relatively harmless, some dominant alleles cause lethal diseases. Those that do are much less common than recessive alleles that have lethal effects. A lethal recessive allele is only lethal when homozygous; it can be passed from one generation to the next by heterozygous carriers because the carriers themselves have normal phenotypes. A lethal dominant allele, however, often causes the death of afflicted individuals before they can mature and reproduce, and in this case the allele is not passed on to future generations.

A lethal dominant allele may be passed on, though, if the lethal disease symptoms first appear after reproductive age. In these cases, the individual may already have transmitted the allele to his or her children. For example, a degenerative disease of the nervous system, called **Huntington's disease**, is caused by a lethal dominant allele that has no obvious phenotypic effect until the individual is about 35 to 45 years old. Once the deterioration of the nervous system begins, it is irreversible and inevitably fatal. As with other dominant traits, a child born to a parent with the Huntington's disease allele has a 50% chance of inheriting the allele and the disorder (see the Punnett square in Figure 14.18). In the United States, this disease afflicts about one in 10,000 people.

At one time, the onset of symptoms was the only way to know if a person had inherited the Huntington's allele, but this is no longer the case. By analyzing DNA samples from a large family with a high incidence of the disorder, geneticists tracked the Huntington's allele to a locus near the tip of chromosome 4, and the gene was sequenced in 1993. This information led to the development of a test that could detect the presence of the Huntington's allele in an individual's genome. (The methods that make such tests possible are discussed in Concepts 19.1 and 19.4.) The availability of this test poses an agonizing dilemma for those with a family history of Huntington's disease. Some individuals may want to be tested for this disease, whereas others may decide it would be too stressful to find out.



Interview with Nancy Wexler: Mapping the gene that causes Huntington's disease

Multifactorial Disorders

The hereditary diseases we have discussed so far are sometimes described as simple Mendelian disorders because they result from abnormality of one or both alleles at a single genetic locus. Many more people are susceptible to diseases that have a multifactorial basis—a genetic component plus a significant environmental influence. Heart disease, diabetes, cancer, alcoholism, certain mental illnesses such as schizophrenia and bipolar disorder, and many other diseases are multifactorial. In these cases, the hereditary component is polygenic. For example, many genes affect cardiovascular health, making some of us more prone than others to heart attacks and strokes. No matter what our genotype, however, our lifestyle has a tremendous effect on phenotype for cardiovascular health and other multifactorial characters. Exercise,

a healthful diet, abstinence from smoking, and an ability to handle stressful situations all reduce our risk of heart disease and some types of cancer.

Genetic Testing and Counseling

Avoiding simple Mendelian disorders is possible when the risk of a particular genetic disorder can be assessed before a child is conceived or during the early stages of the pregnancy. Many hospitals have genetic counselors who can provide information to prospective parents concerned about a family history for a specific disease. Fetal and newborn testing can also reveal genetic disorders.

Counseling Based on Mendelian Genetics and Probability Rules

Consider the case of a hypothetical couple, John and Carol. Each had a brother who died from the same recessively inherited lethal disease. Before conceiving their first child, John and Carol seek genetic counseling to determine the risk of having a child with the disease. From the information about their brothers, we know that both parents of John and both parents of Carol must have been carriers of the recessive allele. Thus, John and Carol are both products of $Aa \times Aa$ crosses, where a symbolizes the allele that causes this particular disease. We also know that John and Carol are not homozygous recessive (aa), because they do not have the disease. Therefore, their genotypes are either AA or Aa .

Given a genotypic ratio of $1 AA : 2 Aa : 1 aa$ for offspring of an $Aa \times Aa$ cross, John and Carol each have a $\frac{2}{3}$ chance of being carriers (Aa). According to the rule of multiplication, the overall probability of their firstborn having the disorder is $\frac{2}{3}$ (the chance that John is a carrier) times $\frac{2}{3}$ (the chance that Carol is a carrier) times $\frac{1}{4}$ (the chance of two carriers having a child with the disease), which equals $\frac{1}{9}$. Suppose that Carol and John decide to have a child—after all, there is an $\frac{8}{9}$ chance that their baby will not have the disorder. If, despite these odds, their child is born with the disease, then we would know that *both* John and Carol are, in fact, carriers (Aa genotype). If both John and Carol are carriers, there is a $\frac{1}{4}$ chance that any subsequent child this couple has will have the disease. The probability is higher for subsequent children because the diagnosis of the disease in the first child established that both parents are carriers, not because the genotype of the first child affects in any way that of future children.

When we use Mendel's laws to predict possible outcomes of matings, it is important to remember that each child represents an independent event in the sense that its genotype is unaffected by the genotypes of older siblings. Suppose that John and Carol have three more children, and *all three* have the hypothetical hereditary disease. There is only one chance in 64 ($\frac{1}{4} \times \frac{1}{4} \times \frac{1}{4}$) that such an outcome will occur. Despite this run of misfortune, the chance that a fourth child of this couple will have the disease remains $\frac{1}{4}$.

Tests for Identifying Carriers

Most children with recessive disorders are born to parents with normal phenotypes. The key to accurately assessing the genetic risk for a particular disease is therefore to find out whether the prospective parents are heterozygous carriers of the recessive allele. For an increasing number of heritable disorders, tests are available that can distinguish individuals of normal phenotype who are dominant homozygotes from those who are heterozygous carriers. There are now tests that can identify carriers of the alleles for Tay-Sachs disease, sickle-cell disease, and the most common form of cystic fibrosis. A program testing for carriers of Tay-Sachs disease that began in the 1980s has successfully reduced the rate of babies born with this disease.

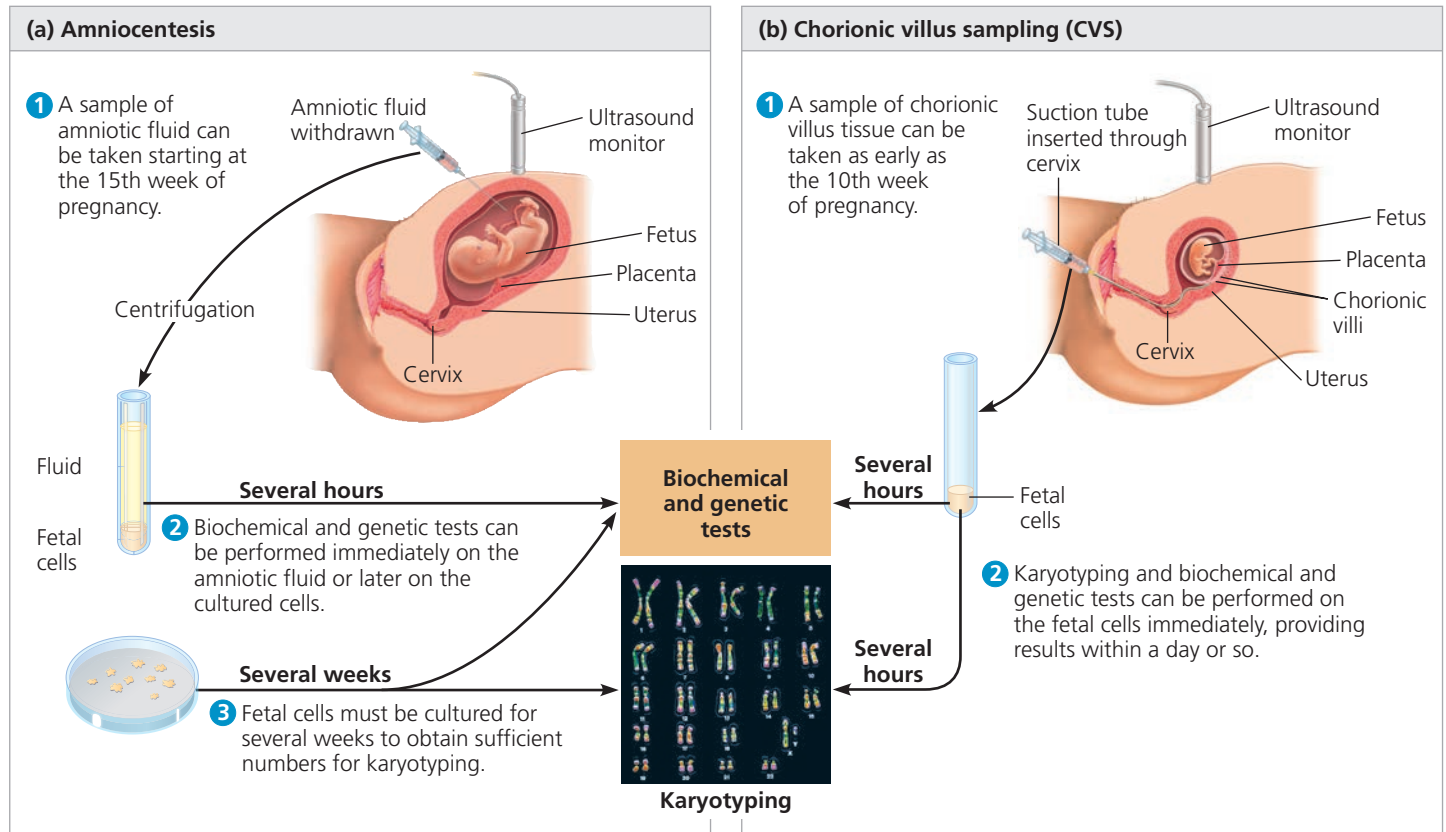
These tests for identifying carriers enable people with family histories of genetic disorders to make informed decisions about having children, including whether to do genetic testing of the fetus, should they decide to become pregnant. The tests also raise other issues: Could carriers be denied health or life insurance or lose the jobs providing those benefits, even though they themselves are healthy? The Genetic Information Nondiscrimination Act, signed into law in the United States in 2008, allays these concerns by prohibiting discrimination in employment or insurance coverage based on genetic test results. A question that remains is whether sufficient genetic counseling is available to help large numbers of individuals understand their genetic test results. Even when test results are clearly understood, affected individuals may still face difficult decisions. Advances in biotechnology offer the potential to reduce human suffering, but along with them come ethical issues that require conscientious deliberation.

Fetal Testing

Suppose a couple expecting a child learns that they are both carriers of the Tay-Sachs allele. One of the tests that can be done to determine whether the developing fetus has Tay-Sachs disease is **amniocentesis**, which can be performed starting at the 15th week of pregnancy (**Figure 14.19a**). In this procedure, a physician inserts a needle into the uterus and extracts about 10 mL of amniotic fluid, the liquid that bathes the fetus. Some genetic disorders can be detected from the presence of certain molecules in the amniotic fluid itself. Tests for other disorders, including Tay-Sachs disease, are performed on the DNA of cells cultured in the laboratory, descendants of fetal cells sloughed off into the amniotic fluid. A karyotype of these cultured cells can also identify certain chromosomal defects (see Figure 13.3).

In an alternative technique called **chorionic villus sampling (CVS)**, a physician inserts a narrow tube through the cervix into the uterus and suctions out a tiny sample of tissue from the placenta, the organ that transmits nutrients and fetal wastes between the fetus and the mother (**Figure 14.19b**). The cells of the chorionic villi of the placenta—the portion sampled—are derived from the

▼ **Figure 14.19 Testing a fetus for genetic disorders.** Biochemical tests may detect substances associated with particular disorders, and genetic testing can detect many genetic abnormalities. Karyotyping shows whether the chromosomes of the fetus are normal in number and appearance.



fetus and have the same genotype and DNA sequence as the new individual. These cells are proliferating rapidly enough to allow karyotyping to be carried out immediately. This rapid analysis represents an advantage over amniocentesis, in which the cells must be cultured for several weeks before karyotyping. Another advantage of CVS is that it can be performed as early as the 10th week of pregnancy.

Medical scientists have also developed methods for isolating fetal cells, or even fetal DNA, that have escaped into the mother's blood. Although very few are present, the cells can be cultured and tested, and the fetal DNA can be analyzed. In 2012, researchers were able to analyze the entire genome of a fetus, comparing sequences of samples obtained from both parents and fetal DNA found in the mother's blood. Cell-free fetal DNA tests and other blood tests are increasingly being used as noninvasive prenatal screening tests for certain disorders; a positive result indicates to the parents that further diagnostic testing, such as amniocentesis or CVS, should be considered.

Imaging techniques allow a physician to examine a fetus directly for major anatomical abnormalities that might not show up in genetic tests. In the *ultrasound* technique, for example, reflected sound waves are used to produce an image of the fetus by a simple noninvasive procedure.

Ultrasound and isolation of fetal cells or DNA from maternal blood pose no known risk to either mother or

fetus, while the other procedures can cause complications in a small percentage of cases. Amniocentesis or CVS for diagnostic testing is generally offered to women over age 35, due to their increased risk of bearing a child with Down syndrome, and may also be offered to younger women if there are known concerns. If the fetal tests reveal a serious disorder like Tay-Sachs, the parents face the difficult choice of either terminating the pregnancy or preparing to care for a child with a genetic disorder, one that might even be fatal. Parental and fetal screening for Tay-Sachs alleles done since 1980 has reduced the number of children born with this incurable disease by 90%. In 2008, the Chinese government initiated a program of fetal testing to detect a harmful genetic blood disorder called β -thalassemia. This effort resulted in a reduction in the rate of this disorder from over 21 births per 1000 in 2008 to just under 13 in 2011.

Newborn Screening

Some genetic disorders can be detected at birth by simple biochemical tests that are now routinely performed in most hospitals in the United States. One common screening program is for phenylketonuria (PKU), a recessively inherited disorder that occurs in about one out of every 10,000–15,000 births in the United States. Children with this disease cannot properly metabolize the amino acid phenylalanine. This

compound and its by-product, phenylpyruvate, can accumulate to toxic levels in the blood, causing severe intellectual disability (mental retardation). However, if PKU is detected in the newborn, a special diet low in phenylalanine will usually allow normal development. (Among many other substances, this diet excludes the artificial sweetener aspartame, which contains phenylalanine.) Unfortunately, few other genetic disorders are treatable at present.

Fetal and newborn screening for serious inherited diseases, tests for identifying carriers, and genetic counseling all rely on the Mendelian model of inheritance. We owe the “gene idea”—the concept of heritable factors transmitted according to simple rules of chance—to the elegant quantitative experiments of Gregor Mendel. The importance of his discoveries was overlooked by most biologists until early in the 20th century, decades after he reported his findings. In the next chapter, you will learn how Mendel’s laws have their physical basis in the behavior of chromosomes during sexual life cycles and how the synthesis of Mendelian genetics and a chromosome theory of inheritance catalyzed progress in genetics.

CONCEPT CHECK 14.4

1. Beth and Tom each have a sibling with cystic fibrosis, but neither Beth nor Tom nor any of their parents have the disease. Calculate the probability that if this couple has a child, the child will have cystic fibrosis. What would be the probability if a test revealed that Tom is a carrier but Beth is not? Explain your answers.
2. **MAKE CONNECTIONS** Explain how the change of a single amino acid in hemoglobin leads to the aggregation of hemoglobin into long fibers. (Review Figures 5.14, 5.18, and 5.19.)
3. Joan was born with six toes on each foot, a dominant trait called polydactyly. Two of her five siblings and her mother, but not her father, also have extra digits. What is Joan’s genotype for the number-of-digits character? Explain your answer. Use D and d to symbolize the alleles for this character.
4. **MAKE CONNECTIONS** In Table 14.1, note the phenotypic ratio of the dominant to recessive trait in the F_2 generation for the monohybrid cross involving flower color. Then determine the phenotypic ratio for the offspring of the second-generation couple in Figure 14.15b. What accounts for the difference in the two ratios?

For suggested answers, see Appendix A.

14 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 14.1

Mendel used the scientific approach to identify two laws of inheritance
(pp. 320–326)



- Gregor Mendel formulated a theory of inheritance based on experiments with garden peas, proposing that parents pass on to their offspring discrete genes that retain their identity through generations. This theory includes two “laws.”
- The **law of segregation** states that genes have alternative forms, or **alleles**. In a diploid organism, the two alleles of a gene segregate (separate) during meiosis and gamete formation; each sperm or egg carries only one allele of each pair. This law explains the 3:1 ratio of F_2 phenotypes observed when **monohybrids** self-pollinate. Each organism inherits one allele for each gene from each parent. In **heterozygotes**, the two alleles are different; expression of the **dominant allele** masks the phenotypic effect of the **recessive allele**. **Homozygotes** have identical alleles of a given gene and are therefore **true-breeding**.
- The **law of independent assortment** states that the pair of alleles for a given gene segregates into gametes independently of the pair of alleles for any other gene. In a cross between **dihybrids** (individuals heterozygous for two genes), the offspring have four phenotypes in a 9:3:3:1 ratio.

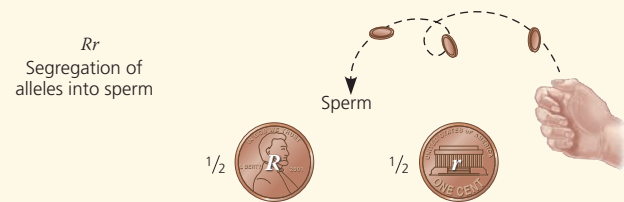
? When Mendel did crosses of true-breeding purple- and white-flowered pea plants, the white-flowered trait disappeared from the F_1 generation but reappeared in the F_2 generation. Use genetic terms to explain why that happened.



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CONCEPT 14.2

Probability laws govern Mendelian inheritance
(pp. 326–328)



- The **multiplication rule** states that the probability of two or more events occurring together is equal to the product of the individual probabilities of the independent single events. The **addition rule** states that the probability of an event that can occur in two or more independent, mutually exclusive ways is the sum of the individual probabilities.
- The rules of probability can be used to solve complex genetics problems. A dihybrid or other multicharacter cross is equivalent to two or more independent monohybrid crosses occurring simultaneously. In calculating the chances of the various offspring genotypes from such crosses, each character is first considered separately and then the individual probabilities are multiplied.

DRAW IT Redraw the Punnett square on the right side of Figure 14.8 as two smaller monohybrid Punnett squares, one for each gene. Below each square, list the fractions of each phenotype produced. Use the rule of multiplication to compute the overall fraction of each possible dihybrid phenotype. What is the phenotypic ratio?

CONCEPT 14.3

Inheritance patterns are often more complex than predicted by simple Mendelian genetics (pp. 328–333)

- Extensions of Mendelian genetics for a single gene:

Relationship among alleles of a single gene	Description	Example
Complete dominance of one allele	Heterozygous phenotype same as that of homozygous dominant	PP Pp
Incomplete dominance of either allele	Heterozygous phenotype intermediate between the two homozygous phenotypes	$C^R C^R$ $C^R C^W$ $C^W C^W$
Codominance	Both phenotypes expressed in heterozygotes	$I^A I^B$
Multiple alleles	In the population, some genes have more than two alleles	ABO blood group alleles I^A, I^B, i
Pleiotropy	One gene affects multiple phenotypic characters	Sickle-cell disease

- Extensions of Mendelian genetics for two or more genes:

Relationship among two or more genes	Description	Example
Epistasis	The phenotypic expression of one gene affects the expression of another gene	$BbEe$ $\times$ $BbEe$ 9 : 3 : 4
Polygenic inheritance	A single phenotypic character is affected by two or more genes	$AaBbCc$ $\times$ $AaBbCc$

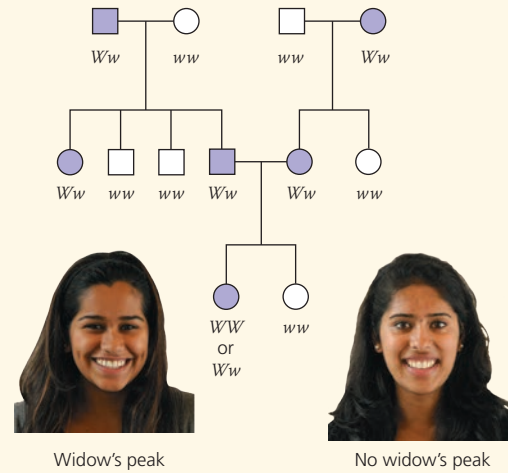
- The expression of a genotype can be affected by environmental influences, resulting in a range of phenotypes. Polygenic characters that are also influenced by the environment are called **multifactorial** characters.
- An organism's overall phenotype, including its physical appearance, internal anatomy, physiology, and behavior, reflects its overall genotype and unique environmental history. Even in more complex inheritance patterns, Mendel's fundamental laws still apply.

? Which genetic relationships listed in the first column of the two tables above are demonstrated by the inheritance pattern of the ABO blood group alleles? For each genetic relationship, explain why this inheritance pattern is or is not an example.

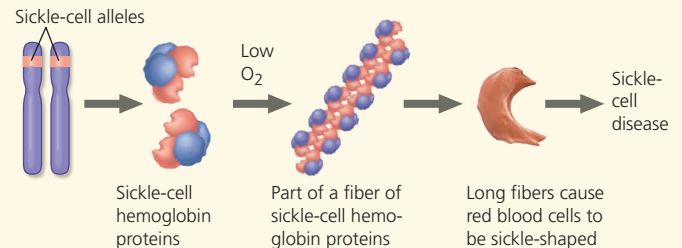
CONCEPT 14.4

Many human traits follow Mendelian patterns of inheritance (pp. 334–340)

- Analysis of family **pedigrees** can be used to deduce the possible genotypes of individuals and make predictions about future offspring. Such predictions are statistical probabilities rather than certainties.



- Many genetic disorders are inherited as simple recessive traits. Most affected (homozygous recessive) individuals are children of phenotypically normal, heterozygous **carriers**.
- The sickle-cell allele has probably persisted for evolutionary reasons: Homozygotes have sickle-cell disease, but heterozygotes have an advantage because one copy of the sickle-cell allele reduces both the frequency and severity of malaria attacks.



- Lethal dominant alleles are eliminated from the population if affected people die before reproducing. Nonlethal dominant alleles and lethal ones that strike relatively late in life can be inherited in a Mendelian way.
- Many human diseases are multifactorial—that is, they have both genetic and environmental components and do not follow simple Mendelian inheritance patterns.
- Using family histories, genetic counselors help couples determine the probability that their children will have genetic disorders. Genetic testing of prospective parents to reveal whether they are carriers of recessive alleles associated with specific disorders has become widely available. Blood tests can screen for certain disorders in a fetus. **Amniocentesis** and **chorionic villus sampling** can indicate whether a suspected genetic disorder is present in a fetus. Other genetic tests can be performed after birth.

? Both members of a couple know that they are carriers of the cystic fibrosis allele. None of their three children has cystic fibrosis, but any one of them might be a carrier. The couple would like to have a fourth child but are worried that he or she would very likely have the disease, since the first three do not. What would you tell the couple? Would it remove some uncertainty from their prediction if they could find out whether the three children are carriers?

TIPS FOR GENETICS PROBLEMS

- Write down symbols for the alleles. (These may be given in the problem.) When represented by single letters, the dominant allele is uppercase and the recessive is lowercase.
- Write down the possible genotypes, as determined by the phenotype.
 - If the phenotype is that of the dominant trait (for example, purple flowers), then the genotype is either homozygous dominant or heterozygous (PP or Pp in this example).
 - If the phenotype is that of the recessive trait, the genotype must be homozygous recessive (for example, pp).
 - If the problem says "true-breeding," the genotype is homozygous.
- Determine what the problem is asking for. If asked to do a cross, write it out in the form [genotype] $\times$ [genotype], using the alleles you've decided on.
- To figure out the outcome of a cross, set up a Punnett square.
 - Put the gametes of one parent at the top and those of the other on the left. To determine the allele(s) in each gamete for a given genotype, set up a systematic way to list all the possibilities. (Remember, each gamete has one allele of each gene.) Note that there are 2^n possible types of gametes, where n is the number of gene loci that are heterozygous. For example, an individual with genotype $AaBbCc$ would produce $2^3 = 8$ types of gametes. Write the genotypes of the gametes in circles above the columns and to the left of the rows.
 - Fill in the Punnett square as if each possible sperm were fertilizing each possible egg, making all of the possible offspring. In a cross of $AaBbCc \times AaBbCc$, for example, the Punnett square would have 8 columns and 8 rows, so there are 64 different offspring; you would know the genotype of each and thus the phenotype. Count genotypes and phenotypes to obtain the genotypic and phenotypic ratios. Because the Punnett square is so large, this method is not the most efficient. See tip 5.
- You can use the rules of probability if a Punnett square would be too big. (For example, see the question at the end of the summary for Concept 14.2 and question 7 below.) You can consider each gene separately (see the section Solving Complex Genetics Problems with the Rules of Probability in Concept 14.2).
- If the problem gives you the phenotypic ratios of offspring but not the genotypes of the parents in a given cross, the phenotypes can help you deduce the parents' unknown genotypes.
 - For example, if $\frac{1}{2}$ of the offspring have the recessive phenotype and $\frac{1}{2}$ the dominant, you know that the cross was between a heterozygote and a homozygous recessive.
 - If the ratio is 3:1, the cross was between two heterozygotes.
 - If two genes are involved and you see a 9:3:3:1 ratio in the offspring, you know that each parent is heterozygous for both genes. Caution: Don't assume that the reported numbers will exactly equal the predicted ratios. For example, if there are 13 offspring with the dominant trait and 11 with the recessive, assume that the ratio is one dominant to one recessive.
- For pedigree problems, use the tips in Figure 14.15 and below to determine what kind of trait is involved.
 - If parents without the trait have offspring with the trait, the trait must be recessive and the parents both carriers.
 - If the trait is seen in every generation, it is most likely dominant (see the next possibility, though).
 - If both parents have the trait, then in order for it to be recessive, all offspring must show the trait.
 - To determine the likely genotype of a certain individual in a pedigree, first label the genotypes of all the family members you can. Even if some of the genotypes are incomplete, label what you do know. For example, if an individual has the dominant phenotype, the genotype must be AA or Aa ; you can write this as $A-$. Try different possibilities to see which fits the results. Use the rules of probability to calculate the probability of each possible genotype being the correct one.

TEST YOUR UNDERSTANDING

- DRAW IT** Two pea plants heterozygous for the characters of pod color and pod shape are crossed. Draw a Punnett square to determine the phenotypic ratios of the offspring.
- A man with type A blood marries a woman with type B blood. Their child has type O blood. What are the genotypes of these three individuals? What genotypes, and in what frequencies, would you expect in future offspring from this marriage?
- A man has two copies of the defective gene for Huntington's disease. His wife is not a carrier. What is the probability that their daughter will have the disease?
- DRAW IT** A pea plant heterozygous for inflated pods (Ii) is crossed with a plant homozygous for constricted pods (ii). Draw a Punnett square for this cross to predict genotypic and phenotypic ratios. Assume that pollen comes from the ii plant.
- Flower position, stem length, and seed shape are three characters that Mendel studied. Each is controlled by an independently assorting gene and has dominant and recessive expression as indicated in Table 14.1. If a plant that is heterozygous for all three characters is allowed to self-fertilize, what proportion of the offspring would you expect to be each of the following? (Note: Use the rules of probability instead of a huge Punnett square.)



PRACTICE TEST
goo.gl/iAsvGL

- homozygous for the three dominant traits
 - homozygous for the three recessive traits
 - heterozygous for all three characters
 - homozygous for axial and tall, heterozygous for seed shape
- Hemochromatosis is an inherited disease caused by a recessive allele. If a woman and her husband, who are both carriers, have three children, what is the probability of each of the following?
 - All three children are of normal phenotype.
 - One or more of the three children have the disease.
 - All three children have the disease.
 - At least one child is phenotypically normal.
 (Note: It will help to remember that the probabilities of all possible outcomes always add up to 1.)
 - The genotype of F_1 individuals in a tetrahybrid cross is $AaBbCcDd$. Assuming independent assortment of these four genes, what are the probabilities that F_2 offspring will have the following genotypes?
 - $aabbccdd$
 - $AaBbCcDd$
 - $AABBCCDD$
 - $AaBBccDd$
 - $AaBBCCdd$

8. What is the probability that each of the following pairs of parents will produce the indicated offspring? (Assume independent assortment of all gene pairs.)

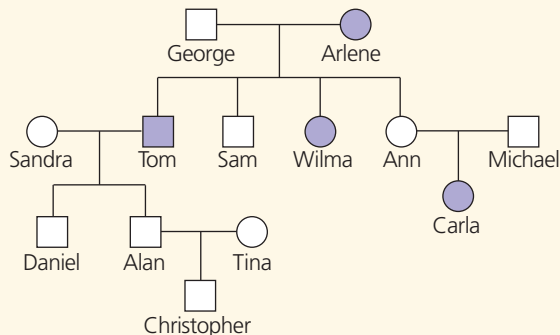
- (a) $AABBCC \times aabbcc \rightarrow AaBbCc$
 (b) $AABbCc \times AaBbCc \rightarrow AAbbCC$
 (c) $AaBbCc \times AaBbCc \rightarrow AaBbCc$
 (d) $aaBbCC \times AABbcc \rightarrow AaBbCc$

9. Karen and Steve each have a sibling with sickle-cell disease. Neither Karen nor Steve nor any of their parents have the disease, and none of them have been tested to see if they carry the sickle-cell allele. Based on this incomplete information, calculate the probability that if this couple has a child, the child will have sickle-cell disease.

10. In 1981, a stray black cat with unusual rounded, curled-back ears was adopted by a family in California. Hundreds of descendants of the cat have since been born, and cat fanciers hope to develop the curl cat into a show breed. Suppose you owned the first curl cat and wanted to develop a true-breeding variety. How would you determine whether the curl allele is dominant or recessive? How would you obtain true-breeding curl cats? How could you be sure they are true-breeding?



11. In tigers, a recessive allele of a particular gene causes both an absence of fur pigmentation (a white tiger) and a cross-eyed condition. If two phenotypically normal tigers that are heterozygous at this locus are mated, what percentage of their offspring will be cross-eyed? What percentage of cross-eyed tigers will be white?
12. In maize (corn) plants, a dominant allele I inhibits kernel color, while the recessive allele i permits color when homozygous. At a different locus, the dominant allele P causes purple kernel color, while the homozygous recessive genotype pp causes red kernels. If plants heterozygous at both loci are crossed, what will be the phenotypic ratio of the offspring?
13. The pedigree below traces the inheritance of alkaptonuria, a biochemical disorder. Affected individuals, indicated here by the colored circles and squares, are unable to metabolize a substance called alkapton, which colors the urine and stains body tissues. Does alkaptonuria appear to be caused by a dominant allele or by a recessive allele? Fill in the genotypes of the individuals whose genotypes can be deduced. What genotypes are possible for each of the other individuals?



14. Imagine that you are a genetic counselor, and a couple planning to start a family comes to you for information. Mary's brother has albinism. Her husband, John, has been tested and has not been found to be a carrier. What is the probability that

Mary is a carrier? What is the probability that Mary and John will have a baby with albinism?

15. **EVOLUTION CONNECTION** Natural selection eliminates individuals with unfavorable phenotypes from a population. However, a number of harmful mutant alleles, particularly in heterozygous individuals, are retained in a gene pool. How may this provide an advantage to the heterozygotes?
16. **SCIENTIFIC INQUIRY** You are handed a mystery pea plant with tall stems and axial flowers and asked to determine its genotype as quickly as possible. You know that the allele for tall stems (T) is dominant to that for dwarf stems (t) and that the allele for axial flowers (A) is dominant to that for terminal flowers (a).
 (a) Identify all the possible genotypes for your mystery plant.
 (b) Describe the one cross you would do, out in your garden, to determine the exact genotype of your mystery plant.
 (c) While waiting for the results of your cross, you predict the results for each possible genotype listed in part a. Explain how you do this and why this is not called "performing a cross."
 (d) Explain how the results of your cross and your predictions will help you learn the genotype of your mystery plant.
17. **WRITE ABOUT A THEME: INFORMATION** The continuity of life is based on heritable information in the form of DNA. In a short essay (100–150 words), explain how the passage of genes from parents to offspring, in the form of particular alleles, ensures perpetuation of parental traits in offspring and, at the same time, genetic variation among offspring. Use genetic terms in your explanation.

18. SYNTHESIZE YOUR KNOWLEDGE



Just for fun, imagine that "shirt-stripping" is a phenotypic character caused by a single gene. Construct a genetic explanation for the appearance of the family in the above photograph, consistent with their "shirt phenotypes." Include in your answer the presumed allele combinations for "shirt-stripping" in each family member. Identify the inheritance pattern shown by the child.

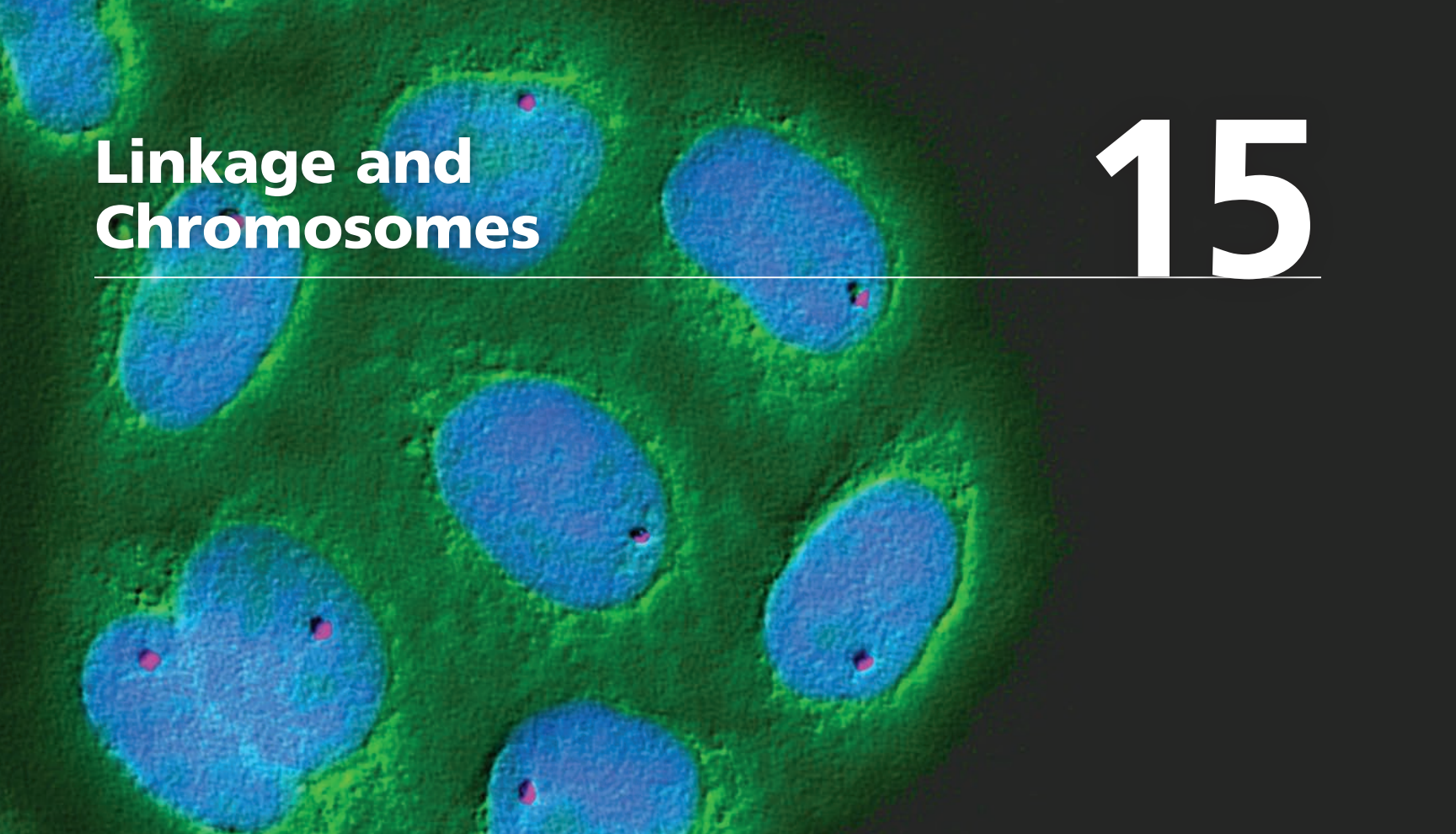
For selected answers, see Appendix A.



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Linkage and Chromosomes

15



▲ **Figure 15.1** Where in the cell are Mendel's hereditary factors located?

KEY CONCEPTS

- 15.1** Morgan showed that Mendelian inheritance has its physical basis in the behavior of chromosomes: *scientific inquiry*
- 15.2** Sex-linked genes exhibit unique patterns of inheritance
- 15.3** Linked genes tend to be inherited together because they are located near each other on the same chromosome
- 15.4** Alterations of chromosome number or structure cause some genetic disorders
- 15.5** Some inheritance patterns are exceptions to standard Mendelian inheritance



Locating Genes Along Chromosomes

Today, we know that genes—Mendel's "factors"—are segments of DNA located along chromosomes. We can see the location of a particular gene by staining with a fluorescent dye that binds specifically to the sequences of that gene. For example, in **Figure 15.1**, the *Pem* gene (in purple) is shown to be localized in the cell nucleus (stained blue), and the cytoplasm is stained green. However, Gregor Mendel's "hereditary factors" were purely an abstract concept when he proposed their existence in 1860. At that time, no cellular structures had been identified that could house these imaginary units, and most biologists were skeptical about Mendel's proposed laws of inheritance.

Using improved techniques of microscopy, cytologists worked out the process of mitosis in 1875 and meiosis in the 1890s. (See the drawing of mitosis published by German biologist Walther Flemming in 1882.) Cytology and genetics converged as biologists began to see parallels between the behavior of Mendel's proposed hereditary factors during sexual life cycles and the behavior of chromosomes: As shown in **Figure 15.2**, chromosomes and genes are both present in pairs in diploid cells, and homologous chromosomes separate and alleles segregate during the process of meiosis. After meiosis, fertilization restores the paired condition for both chromosomes and genes. Around 1902, Walter S. Sutton, Theodor Boveri, and others

◀ Drawing of mitosis (Flemming, 1882)

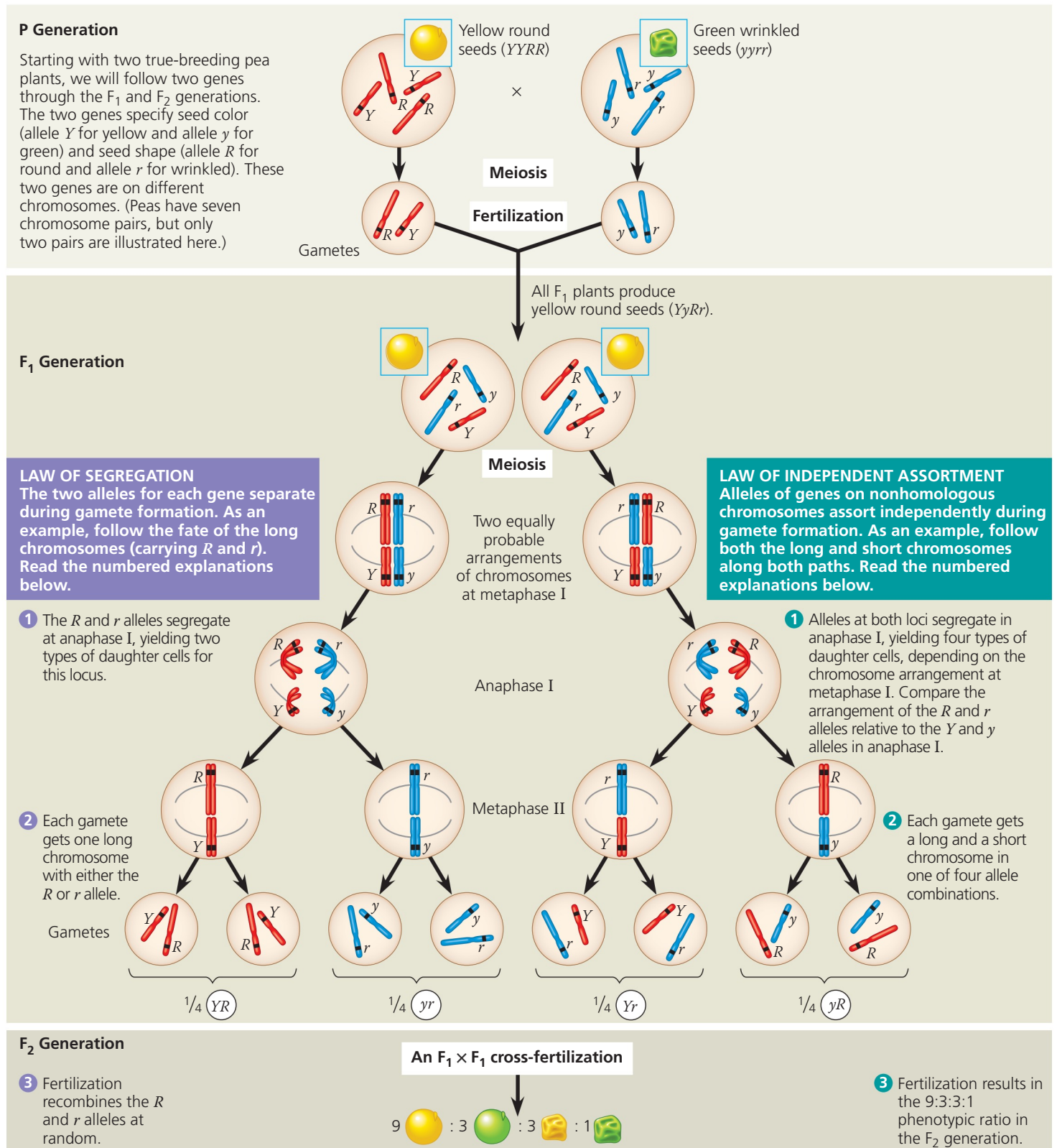
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Get Ready for This Chapter

▼ Figure 15.2 The chromosomal basis of Mendel's laws. Here we correlate the results of one of Mendel's dihybrid crosses (see Figure 14.8) with the behavior of chromosomes during meiosis (see Figure 13.8). The arrangement of chromosomes at metaphase I of meiosis and their movement during anaphase I account for, respectively, the independent assortment and segregation of the alleles for seed color and shape. Each cell that undergoes meiosis in an F_1 plant produces two kinds of gametes. If we count the results for all cells, however, each F_1 plant produces equal numbers of all four kinds of gametes because the alternative chromosome arrangements at metaphase I are equally likely.

MP3 Tutor: The Chromosomal Basis of Inheritance
Animation: The Chromosomal Basis of Independent Assortment



? If you crossed an F_1 plant with a plant that was homozygous recessive for both genes ($yyrr$), how would the phenotypic ratio of the offspring compare with the 9:3:3:1 ratio seen here?

independently noted these parallels and began to develop the **chromosome theory of inheritance**. According to this theory, Mendelian genes have specific loci (positions) along chromosomes, and it is the chromosomes that undergo segregation and independent assortment.

As you can see in Figure 15.2, the separation of homologs during anaphase I accounts for the segregation of the two alleles of a gene into separate gametes, and the random arrangement of chromosome pairs at metaphase I accounts for independent assortment of the alleles for two or more genes located on different homolog pairs. This figure traces the same dihybrid pea cross you learned about in Figure 14.8. By carefully studying Figure 15.2, you can see how the behavior of chromosomes during meiosis in the F_1 generation and subsequent random fertilization give rise to the F_2 phenotypic ratio observed by Mendel.

In correlating the behavior of chromosomes with that of genes, this chapter will extend what you learned in the past two chapters. First, we'll describe evidence from the fruit fly that strongly supported the chromosome theory. (Although this theory made a lot of sense, it still required experimental evidence.) Next, we'll explore the chromosomal basis for the transmission of genes from parents to offspring, including what happens when two genes are linked on the same chromosome. Finally, we will discuss some important exceptions to the standard mode of inheritance.

CONCEPT 15.1

Morgan showed that Mendelian inheritance has its physical basis in the behavior of chromosomes: scientific inquiry

The first solid evidence associating a specific gene with a specific chromosome came early in the 1900s from the work of Thomas Hunt Morgan, an experimental embryologist at Columbia University. Although Morgan was initially skeptical about both Mendelian genetics and the chromosome theory, his early experiments provided convincing evidence that chromosomes are indeed the location of Mendel's heritable factors.

Morgan's Choice of Experimental Organism

Many times in the history of biology, important discoveries have come to those insightful or lucky enough to choose an experimental organism suitable for the research problem being tackled. Mendel chose the garden pea because a number of distinct varieties were available. For his work, Morgan selected a species of fruit fly, *Drosophila melanogaster*, a common insect that feeds on the fungi growing on fruit. Fruit flies are prolific breeders; a single mating will produce hundreds of offspring, and a new generation can be bred

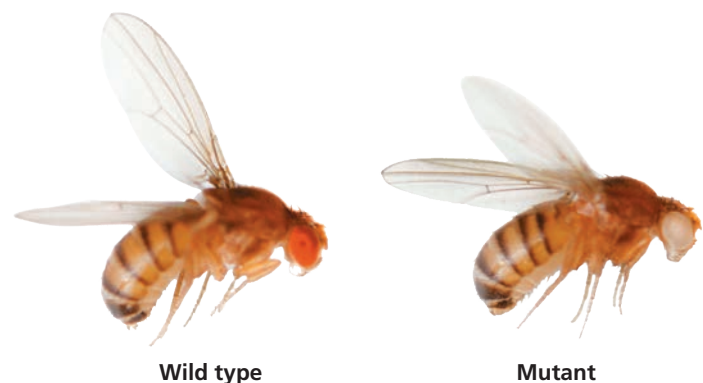
every two weeks. Morgan's laboratory began using this convenient organism for genetic studies in 1907 and soon became known as "the fly room."

Another advantage of the fruit fly is that it has only four pairs of chromosomes, which are easily distinguishable with a light microscope. There are three pairs of autosomes and one pair of sex chromosomes. Female fruit flies have a pair of homologous X chromosomes, and males have one X chromosome and one Y chromosome.

While Mendel could readily obtain different pea varieties from seed suppliers, Morgan was probably the first person to want different varieties of the fruit fly. He faced the tedious task of carrying out many matings and then microscopically inspecting large numbers of offspring in search of naturally occurring variant individuals. After many months of this, he complained, "Two years' work wasted. I have been breeding those flies for all that time and I've got nothing out of it." Morgan persisted, however, and was finally rewarded with the discovery of a single male fly with white eyes instead of the usual red. The phenotype for a character most commonly observed in natural populations, such as red eyes in *Drosophila*, is called the **wild type** (Figure 15.3). Traits that are alternatives to the wild type, such as white eyes in *Drosophila*, are called *mutant phenotypes* because they are due to alleles assumed to have originated as changes, or mutations, in the wild-type allele.

Morgan and his students invented a notation for symbolizing alleles in *Drosophila* that is still widely used for fruit flies. For a given character in flies, the gene takes its symbol from the first mutant (non-wild type) discovered. Thus, the allele for white eyes in *Drosophila* is symbolized by w . A superscript + identifies the allele for the wild-type trait: w^+ for the allele for red eyes, for example. Over the years, a variety of gene notation systems have been developed for different organisms. For example, human genes are usually written in all capitals, such as *HTT* for the gene involved in Huntington's disease. (Alleles may use more than one letter, as this one does.)

▼ **Figure 15.3 Morgan's first mutant.** Wild-type *Drosophila* flies have red eyes (left). Among his flies, Morgan discovered a mutant male with white eyes (right). This variation made it possible for Morgan to trace a gene for eye color to a specific chromosome.



Correlating Behavior of a Gene's Alleles with Behavior of a Chromosome Pair

Morgan mated his white-eyed male fly with a red-eyed female. All the F_1 offspring had red eyes, suggesting that the wild-type allele is dominant. When Morgan bred the F_1 flies to each other, he observed the classical 3:1 phenotypic ratio among the F_2 offspring. However, there was a surprising additional result: The white-eye trait showed up only in males. All the F_2 females had red eyes, while half the males had red eyes and half had white eyes. Therefore, Morgan concluded that somehow a fly's eye color was linked to its sex. (If the eye color gene were unrelated to sex, half of the white-eyed flies would have been female.)

Recall that a female fly has two X chromosomes (XX), while a male fly has an X and a Y (XY). The correlation between the trait of white eye color and the male sex of the affected F_2 flies suggested to Morgan that the gene involved in his white-eyed mutant was located exclusively on the X chromosome, with no corresponding allele present on the Y chromosome. His reasoning can be followed in **Figure 15.4**. For a male, a single copy of the mutant allele would confer white eyes; since a male has only one X chromosome, there can be no wild-type allele (w^+) present to mask the recessive allele. However, a female could have white eyes only if both her X chromosomes carried the recessive mutant allele (w). This was impossible for the F_2 females in Morgan's experiment because all the F_1 fathers had red eyes, so each F_2 female received a w^+ allele on the X chromosome inherited from her father.

Morgan's finding of the correlation between a particular trait and an individual's sex provided support for the chromosome theory of inheritance: namely, that a specific gene is carried on a specific chromosome (in this case, an eye color gene on the X chromosome). In addition, Morgan's work indicated that genes located on a sex chromosome exhibit unique inheritance patterns, which we will discuss in the next section. Recognizing the importance of Morgan's early work, many bright students were attracted to his fly room.

CONCEPT CHECK 15.1

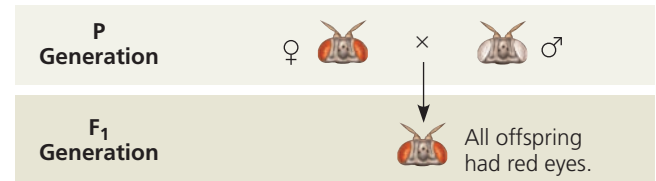
1. Which one of Mendel's laws describes the inheritance of alleles for a single character? Which law relates to the inheritance of alleles for two characters in a dihybrid cross?
2. **MAKE CONNECTIONS** ▶ Review the description of meiosis (see Figure 13.8) and Mendel's laws of segregation and independent assortment (see Concept 14.1). What is the physical basis for each of Mendel's laws?
3. **WHAT IF?** ▶ Propose a possible reason that the first naturally occurring mutant fruit fly Morgan saw involved a gene on a sex chromosome and was found in a male.

For suggested answers, see Appendix A.

Figure 15.4

Inquiry In a cross between a wild-type female fruit fly and a mutant white-eyed male, what color eyes will the F_1 and F_2 offspring have?

Experiment Thomas Hunt Morgan wanted to analyze the behavior of two alleles of a fruit fly eye color gene. In crosses similar to those done by Mendel with pea plants, Morgan and his colleagues mated a wild-type (red-eyed) female with a mutant white-eyed male.

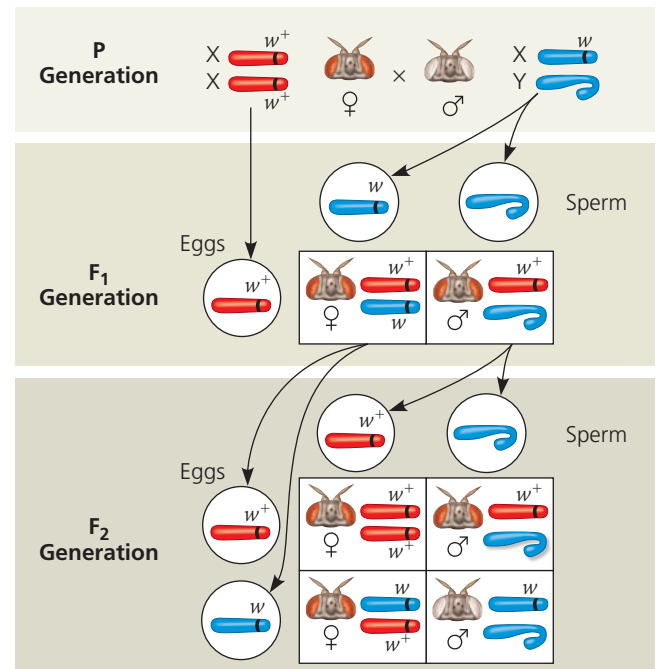


Morgan then bred an F_1 red-eyed female to an F_1 red-eyed male to produce the F_2 generation.

Results The F_2 generation showed a typical Mendelian ratio of 3 red-eyed flies: 1 white-eyed fly. However, all white-eyed flies were males; no females displayed the white-eye trait.



Conclusion All F_1 offspring had red eyes, so the mutant white-eye trait (w) must be recessive to the wild-type red-eye trait (w^+). Since the recessive trait—white eyes—was expressed only in males in the F_2 generation, Morgan deduced that this eye color gene is located on the X chromosome and that there is no corresponding locus on the Y chromosome.



Data from T. H. Morgan, Sex-limited inheritance in *Drosophila*, *Science* 32:120–122 (1910).

Instructors: A related Experimental Inquiry Tutorial can be assigned in MasteringBiology.

WHAT IF? ▶ Suppose this eye color gene were located on an autosome. Predict the phenotypes (including gender) of the F_2 flies in this hypothetical cross. (Hint: Draw a Punnett square.)

CONCEPT 15.2

Sex-linked genes exhibit unique patterns of inheritance

As you just learned, Morgan's discovery of a trait (white eyes) that correlated with the sex of flies was a key episode in the development of the chromosome theory of inheritance. Because the identity of the sex chromosomes in an individual could be inferred by observing the sex of the fly, the behavior of the two members of the pair of sex chromosomes could be correlated with the behavior of the two alleles of the eye color gene. In this section, we'll take a closer look at the role of sex chromosomes in inheritance.

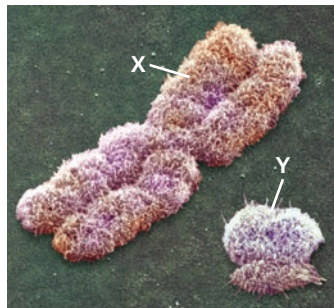
The Chromosomal Basis of Sex

Although sex has traditionally been described as a pair of binary categories, we are coming to understand that sex classifications may be less distinct. Here, we use the term sex to mean the classification into a group with a shared set of anatomical and physiological traits. (The term *gender*, previously used as a synonym of sex, is now more often used to refer to an individual's own cultural experience of identifying as male, female, or otherwise.) In this sense, sex is determined largely by chromosomes.

Humans and other mammals have two types of sex chromosomes, designated X and Y. The Y chromosome is much smaller than the X chromosome (Figure 15.5). A person who inherits two X chromosomes, one from each parent, usually develops anatomy we associate with the "female" sex, while "male" properties are associated with the inheritance of one X chromosome and one Y chromosome (Figure 15.6a). Short segments at either end of the Y chromosome are the only regions that are homologous with regions on the X. These homologous regions allow the X and Y chromosomes in males to pair and behave like homologs during meiosis in the testes.

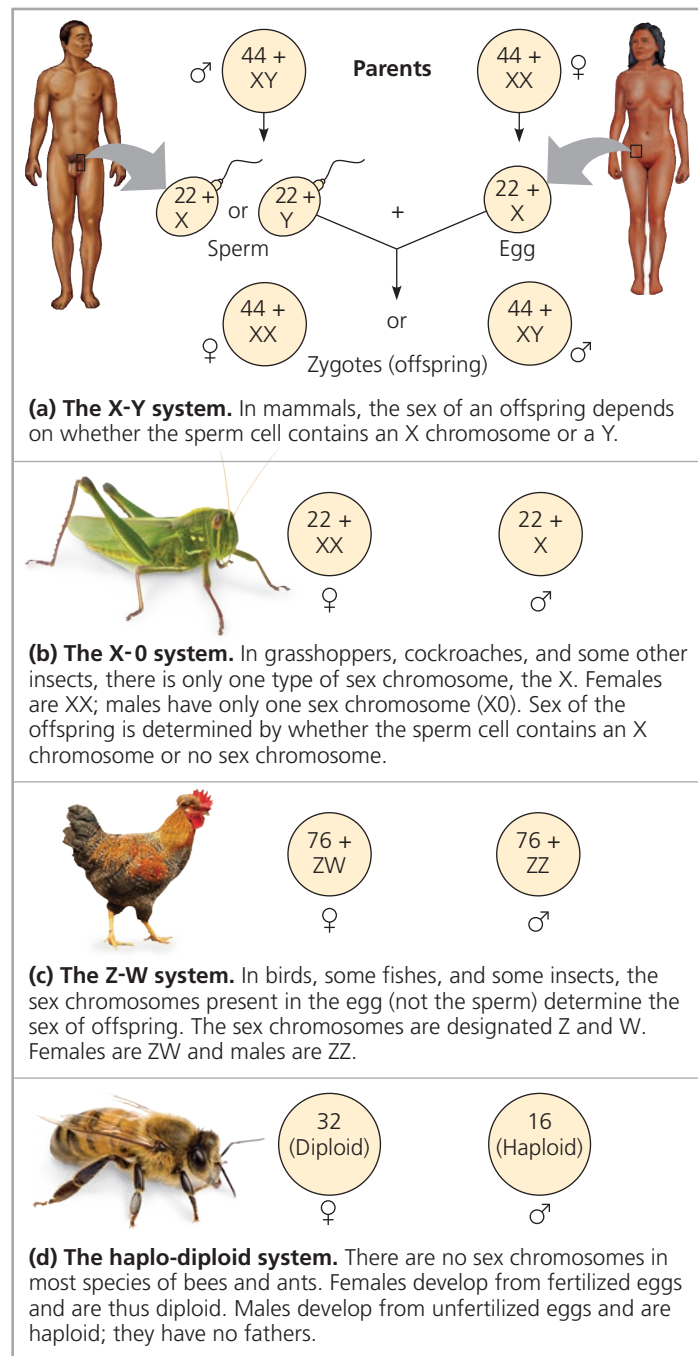
In mammalian testes and ovaries, the two sex chromosomes segregate during meiosis. Each egg receives one X chromosome. In contrast, sperm fall into two categories: Half the sperm cells a male produces receive an X chromosome, and half receive a Y chromosome. We can trace the sex of each offspring to the events of conception: If a sperm cell bearing an X chromosome fertilizes an egg, the zygote is XX, a female; if a sperm cell containing a Y chromosome fertilizes an egg, the zygote is XY, a male (see Figure 15.6a). Thus, in general, sex determination is a matter of chance—a fifty-fifty chance. Note that the mammalian X-Y system isn't the only chromosomal system for determining sex. Figure 15.6b–d illustrates three other systems.

✓ **Figure 15.5 Human sex chromosomes.**



✓ **Figure 15.6 Some chromosomal systems of sex**

determination. Numerals indicate the number of autosomes in the species pictured. In *Drosophila*, males are XY, but sex depends on the ratio of the number of X chromosomes to the number of autosome sets, not simply on the presence of a Y chromosome.



In humans, the anatomical signs of sex begin to emerge when the embryo is about 2 months old. Before then, the rudiments of the gonads are generic—they can develop into either testes or ovaries, depending on whether or not a Y chromosome is present, and depending on what genes are active. A gene on the Y chromosome—called *SRY*, for sex-determining region of *Y*—is required for the development of testes. In the absence of *SRY*, the gonads develop into ovaries, even in an XY embryo.

A gene located on either sex chromosome is called a **sex-linked gene**. The human X chromosome contains approximately 1,100 genes, which are called **X-linked genes**, while genes located on the Y chromosome are called *Y-linked genes*. On the human Y chromosome, researchers have identified 78 genes that code for about 25 proteins (some genes are duplicates). About half of these genes are expressed only in the testis, and some are required for normal testicular functioning and the production of normal sperm. The Y chromosome is passed along virtually intact from a father to all his sons. Because there are so few Y-linked genes, very few disorders are transferred from father to son on the Y chromosome.

The development of female gonads requires a gene called *WNT4* (on chromosome 1, an autosome), which encodes a protein that promotes ovary development. An embryo that is XY but has extra copies of the *WNT4* gene can develop rudimentary female gonads. Overall, sex is determined by the interactions of a network of gene products like these.

The biochemical, physiological, and anatomical features associated with “males” and “females” are turning out to be more complicated than previously thought, with many genes involved in their development. Because of the complexity of this process, many variations exist. Some individuals are born with intermediate sexual (“intersex”) characteristics, or even with anatomical features that do not match an individual’s sense of their own gender (“transgender” individuals). Sex determination is an active area of research that should yield a more sophisticated understanding in years to come.

Inheritance of X-Linked Genes

The fact that males and females inherit a different number of X chromosomes leads to a pattern of inheritance different from that produced by genes located on autosomes. While there are very few Y-linked genes and many help determine

sex, the X chromosomes have genes for many characters unrelated to sex. X-linked genes in humans follow the same pattern of inheritance that Morgan observed for the eye color locus he studied in *Drosophila* (see Figure 15.4). Fathers pass X-linked alleles to all of their daughters but to none of their sons. In contrast, mothers can pass X-linked alleles to both sons and daughters, as shown in **Figure 15.7** for the inheritance of a mild X-linked disorder, red-green color blindness.

If an X-linked trait is due to a recessive allele, a female will express the phenotype only if she is homozygous for that allele. Because males have only one locus, the terms *homozygous* and *heterozygous* lack meaning for describing their X-linked genes; the term *hemizygous* is used in such cases. Any male receiving the recessive allele from his mother will express the trait. For this reason, far more males than females have X-linked recessive disorders. However, even though the chance of a female inheriting a double dose of the mutant allele is much less than the probability of a male inheriting a single dose, there *are* females with X-linked disorders. For instance, color blindness is almost always inherited as an X-linked trait. A color-blind daughter may be born to a color-blind father whose mate is a carrier (see Figure 15.7c). Because the X-linked allele for color blindness is relatively rare, though, the probability that such a man and woman will mate is low.

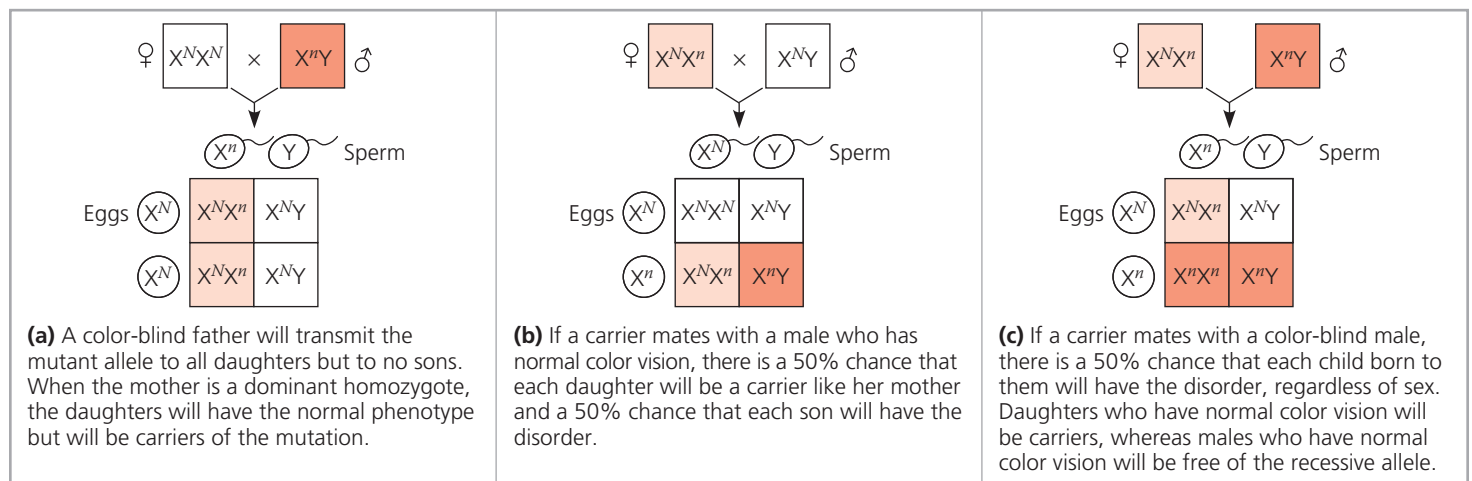
A number of human X-linked disorders are much more serious than color blindness, such as **Duchenne muscular dystrophy**, which affects about one out of 3,500 males born in the United States. The disease is characterized by a progressive weakening of the muscles and loss of coordination. Affected individuals rarely live past their early 20s. Researchers have traced the disorder to the absence of a key muscle protein called dystrophin and have mapped the gene for this protein to a specific locus on the X chromosome.

▼ Figure 15.7 The transmission of X-linked recessive traits. In this diagram, red-green color blindness is used as an example. The superscript *N* represents the dominant allele for normal color vision carried on the X chromosome,

while *n* represents the recessive allele, which has a mutation causing color blindness. White boxes indicate unaffected individuals, light orange boxes indicate carriers, and dark orange boxes indicate color-blind individuals.

? If a color-blind woman married a man who had normal color vision, what would be the probable phenotypes of their children?

Animation: X-Linked Genes



Hemophilia is an X-linked recessive disorder defined by the absence of one or more of the proteins required for blood clotting. When a person with hemophilia is injured, bleeding is prolonged because a firm clot is slow to form. Small cuts in the skin are usually not a problem, but bleeding in the muscles or joints can be painful and can lead to serious damage. In the 1800s, hemophilia was widespread among the royal families of Europe. Queen Victoria of England is known to have passed the allele to several of her descendants. Subsequent intermarriage with royal family members of other nations, such as Spain and Russia, further spread this X-linked trait, and its incidence is well documented in royal pedigrees. A few years ago, new genomic techniques allowed sequencing of DNA from tiny amounts isolated from the buried remains of royal family members. The genetic basis of the mutation, and how it resulted in a nonfunctional blood-clotting factor, is now understood. Today, people with hemophilia are treated as needed with intravenous injections of the protein that is missing.

X Inactivation in Female Mammals

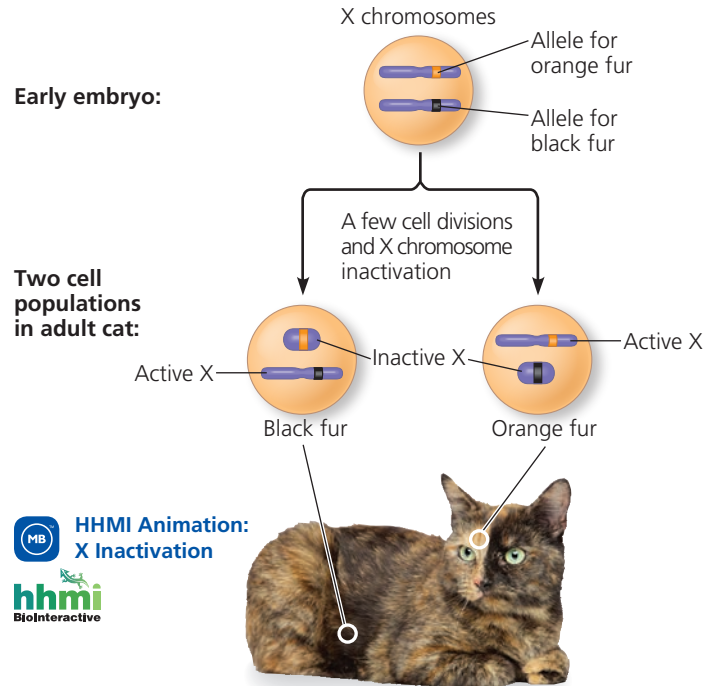
Female mammals, including human females, inherit two X chromosomes—twice the number inherited by males—so you may wonder whether females make twice as many of the proteins encoded by X-linked genes as males. In fact, almost all of one X chromosome in each cell in female mammals becomes inactivated during early embryonic development. As a result, the cells of females and males have the same effective dose (one active copy) of most X-linked genes. The inactive X in each cell of a female condenses into a compact object called a **Barr body** (discovered by Canadian anatomist Murray Barr), which lies along the inside of the nuclear envelope. Most of the genes of the X chromosome that forms the Barr body are not expressed. In the ovaries, however, Barr body chromosomes are reactivated in the cells that give rise to eggs, resulting in every female gamete (egg) having an active X after meiosis.

British geneticist Mary Lyon demonstrated that the selection of which X chromosome will form the Barr body occurs randomly and independently in each embryonic cell present at the time of X inactivation. As a consequence, females consist of a *mosaic* of two types of cells: those with the active X derived from the father and those with the active X derived from the mother. After an X chromosome is inactivated in a particular cell, all mitotic descendants of that cell have the same inactive X. Thus, if a female is heterozygous for a sex-linked trait, about half of her cells will express one allele, while the others will express the alternate allele. **Figure 15.8** shows how this mosaicism results in the patchy coloration of a tortoiseshell cat. In humans, mosaicism can be observed in a recessive X-linked mutation that prevents the development of sweat glands. A woman who is heterozygous for this trait has patches of normal skin and patches of skin lacking sweat glands.

Inactivation of an X chromosome involves modification of the DNA and proteins bound to it called histones, including attachment of methyl groups ($-\text{CH}_3$) to DNA nucleotides.

Figure 15.8 X inactivation and the tortoiseshell cat.

The tortoiseshell gene is on the X chromosome, and the tortoiseshell phenotype requires the presence of two different alleles, one for orange fur and one for black fur. Normally, only females can have both alleles, because only they have two X chromosomes. If a female cat is heterozygous for the tortoiseshell gene, she is tortoiseshell. Orange patches are formed by populations of cells in which the X chromosome with the orange allele is active; black patches have cells in which the X chromosome with the black allele is active. ("Calico" cats also have white areas, which are determined by another gene.)



(The regulatory role of DNA methylation is discussed in Concept 18.2.) A particular region of each X chromosome contains several genes involved in the inactivation process. The two regions, one on each X chromosome, associate briefly with each other in each cell at an early stage of embryonic development. Then one of the genes, called *XIST* (for X-inactive specific transcript), becomes active *only* on the chromosome that will become the Barr body. Multiple copies of the RNA product of this gene apparently attach to the X chromosome on which they are made, eventually almost covering it. Interaction of this RNA with the chromosome initiates X inactivation, and the RNA products of nearby genes help to regulate the process.

CONCEPT CHECK 15.2

1. A white-eyed female *Drosophila* is mated with a red-eyed (wild-type) male, the reciprocal cross of the one shown in Figure 15.4. What phenotypes and genotypes do you predict for the offspring from this cross?
2. Neither Tim nor Rhoda has Duchenne muscular dystrophy, but their firstborn son does. What is the probability that a second child will have the disease? What is the probability if the second child is a boy? A girl?
3. **MAKE CONNECTIONS** Consider what you learned about dominant and recessive alleles in Concept 14.1. If a disorder were caused by a dominant X-linked allele, how would the inheritance pattern differ from what we see for recessive X-linked disorders?

For suggested answers, see Appendix A.

CONCEPT 15.3

Linked genes tend to be inherited together because they are located near each other on the same chromosome

The number of genes in a cell is far greater than the number of chromosomes; in fact, each chromosome (except the Y) has hundreds or thousands of genes. Genes located near each other on the same chromosome tend to be inherited together in genetic crosses; such genes are said to be genetically linked and are called **linked genes**. When geneticists follow linked genes in breeding experiments, the results deviate from those expected from Mendel's law of independent assortment.

Figure 15.9

Inquiry How does linkage between two genes affect inheritance of characters?

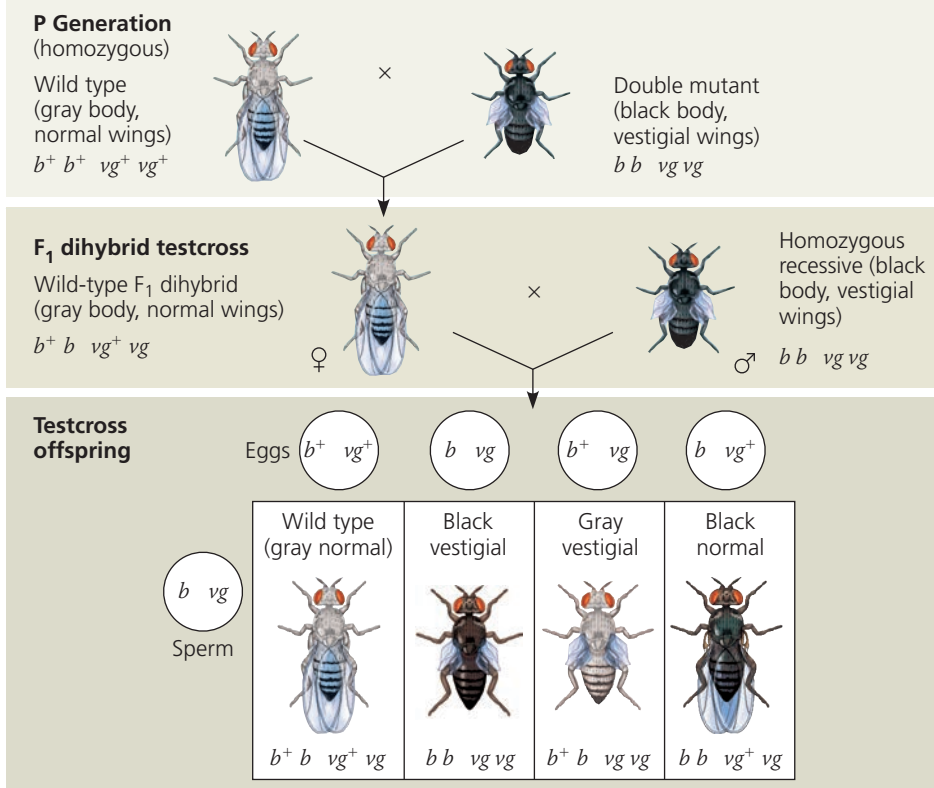
Experiment Morgan wanted to know whether the genes for body color and wing size are genetically linked and, if so, how this affects their inheritance. The alleles for body color are b^+ (gray) and b (black), and those for wing size are vg^+ (normal) and vg (vestigial).

Morgan mated true-breeding P (parental) generation flies—wild-type flies with black, vestigial-winged flies—to produce heterozygous F_1 dihybrids ($b^+ b \quad vg^+ vg$), all of which are wild-type in appearance.

He then mated wild-type F_1 dihybrid females with homozygous recessive males. This testcross will reveal the genotype of the eggs made by the dihybrid female.

The male's sperm contributes only recessive alleles, so the phenotype of the offspring reflects the genotype of the female's eggs.

Note: Although only females (with pointed abdomens) are shown, half the offspring in each class would be males (with rounded abdomens).



Predicted ratios of testcross offspring

- if genes are located on different chromosomes: 1 : 1 : 1 : 1
- if genes are located on the same chromosome and parental alleles are always inherited together: 1 : 1 : 0 : 0

Results

Data from Morgan's experiment: 965 : 944 : 206 : 185

Conclusion Since most offspring had a parental (P generation) phenotype, Morgan concluded that the genes for body color and wing size are genetically linked on the same chromosome. However, the production of a relatively small number of offspring with nonparental phenotypes indicated that some mechanism occasionally breaks the linkage between specific alleles of genes on the same chromosome.

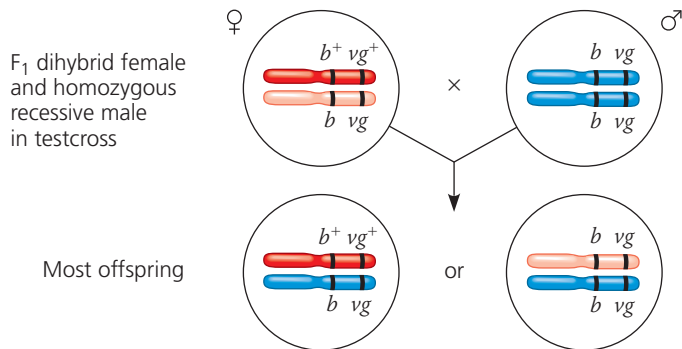
How Linkage Affects Inheritance

To see how linkage between genes affects the inheritance of two different characters, let's examine another of Morgan's *Drosophila* experiments. In this case, the characters are body color and wing size, each with two different phenotypes. Wild-type flies have gray bodies and normal-sized wings. In addition to these flies, Morgan had managed to obtain, through breeding, doubly mutant flies with black bodies and wings much smaller than normal, called vestigial wings. The mutant alleles are recessive to the wild-type alleles, and neither gene is on a sex chromosome. In his investigation of these two genes, Morgan carried out the crosses shown in **Figure 15.9**. The first was a P generation cross to generate F_1 dihybrid flies, and the second was a testcross.

Data from T. H. Morgan and C. J. Lynch, The linkage of two factors in *Drosophila* that are not sex-linked, *Biological Bulletin* 23:174–182 (1912).

WHAT IF? > If the parental (P generation) flies had been true-breeding for gray body with vestigial wings and black body with normal wings, which phenotypic class(es) would be largest among the testcross offspring?

The resulting flies had a much higher proportion of the combinations of traits seen in the P generation flies (called parental phenotypes) than would be expected if the two genes assorted independently. Morgan thus concluded that body color and wing size are usually inherited together in specific (parental) combinations because the genes are linked; they are near each other on the same chromosome:



As you proceed, be sure to keep in mind the distinction between the terms *linked genes* (two or more genes on the same chromosome that tend to be inherited together) and *sex-linked gene* (a single gene on a sex chromosome).

As Figure 15.9 shows, both of the combinations of traits not seen in the P generation (called nonparental phenotypes) were also produced in Morgan's experiments, suggesting that the body color and wing size alleles are not always linked genetically. To understand this conclusion, we need to further explore **genetic recombination**, the production of offspring with combinations of traits that differ from those found in either P generation parent.

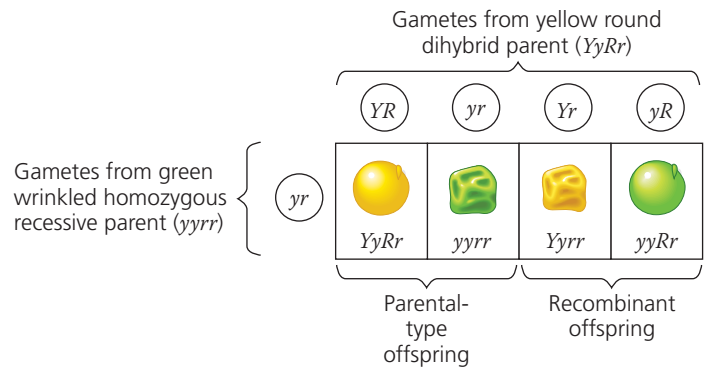
Genetic Recombination and Linkage

Meiosis and random fertilization generate genetic variation among offspring of sexually reproducing organisms due to independent assortment of chromosomes, crossing over in meiosis I, and the possibility of any sperm fertilizing any egg (see Concept 13.4). Here we'll examine the chromosomal basis of recombination of alleles in relation to the genetic findings of Mendel and Morgan.

Recombination of Unlinked Genes: Independent Assortment of Chromosomes

Mendel learned from crosses in which he followed two characters that some offspring have combinations of traits that do not match those of either parent. For example, consider a cross of a dihybrid pea plant with yellow round seeds, heterozygous for both seed color and seed shape ($YyRr$), with a plant homozygous for both recessive alleles (with green wrinkled seeds, $yyrr$). (This cross acts as a testcross because the results will reveal the genotype of the gametes made in

the dihybrid $YyRr$ plant.) Let's represent the cross by the following Punnett square:



Notice in this Punnett square that one-half of the offspring are expected to inherit a phenotype that matches either of the phenotypes of the P (parental) generation originally crossed to produce the F₁ dihybrid (see Figure 15.2). These matching offspring are called **parental types**. But two nonparental phenotypes are also found among the offspring. Because these offspring have new combinations of seed shape and color, they are called **recombinant types**, or **recombinants** for short. When 50% of all offspring are recombinants, as in this example, geneticists say that there is a 50% frequency of recombination. The predicted phenotypic ratios among the offspring are similar to what Mendel actually found in his $YyRr \times yyrr$ crosses.

A 50% frequency of recombination in such testcrosses is observed for any two genes that are located on different chromosomes and thus cannot be linked. The physical basis of recombination between unlinked genes is the random orientation of homologous chromosomes at metaphase I of meiosis, which leads to the independent assortment of the two unlinked genes (see Figure 13.11 and the question in the Figure 15.2 legend).

Recombination of Linked Genes: Crossing Over

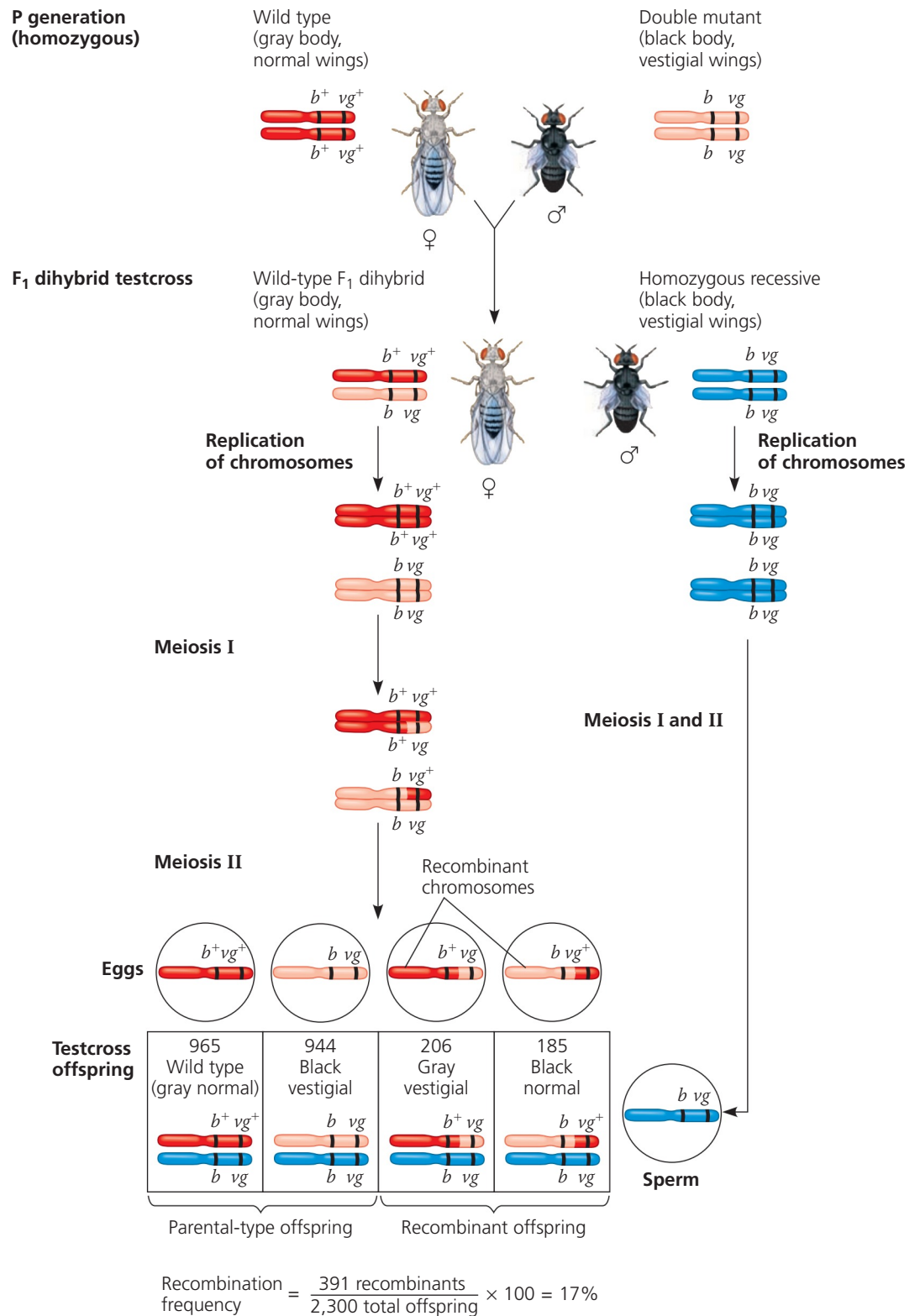
Now, let's explain the results of the *Drosophila* testcross in Figure 15.9. Recall that most of the offspring from the testcross for body color and wing size had parental phenotypes. That suggested that the two genes were on the same chromosome, since the occurrence of parental types with a frequency greater than 50% indicates that the genes are linked. About 17% of offspring, however, were recombinants.

Seeing these results, Morgan proposed that some process must occasionally break the physical connection between specific alleles of genes on the same chromosome. Later experiments showed that this process, now called **crossing over**, accounts for the recombination of linked genes. In crossing over, which occurs while replicated homologous chromosomes are paired during prophase of meiosis I, a set of proteins orchestrates an exchange of corresponding segments of one maternal and one paternal chromatid (see Figure 13.9).

► **Figure 15.10 Chromosomal basis for recombination of linked genes.** In these diagrams re-creating the testcross in Figure 15.9, we track chromosomes as well as genes. The maternal chromosomes (present in the wild-type F_1 dihybrid) are color-coded red and pink to distinguish one homolog from the other before any meiotic crossing over has occurred. Because crossing over between the b^+/b and vg^+/vg loci occurs in some, but not all, egg-producing cells, more eggs with parental-type chromosomes than with recombinant ones are produced in the mating females. Fertilization of the eggs by sperm of genotype $b\ vg$ gives rise to some recombinant offspring. The recombination frequency is the percentage of recombinant flies in the total pool of offspring.

DRAW IT ► Suppose, as in the question at the bottom of Figure 15.9, the parental (P generation) flies were true-breeding for gray body with vestigial wings and black body with normal wings. Draw the chromosomes in each of the four possible kinds of eggs from an F_1 female, and label each chromosome as “parental” or “recombinant.”

 **Animation: Linked Genes and Crossing Over**



In effect, when a single crossover occurs, end portions of two nonsister chromatids trade places.

Figure 15.10 shows how crossing over in a dihybrid female fly resulted in recombinant eggs and ultimately recombinant offspring in Morgan’s testcross. Most eggs had a chromosome with either the $b^+ vg^+$ or $b\ vg$ parental genotype, but some had a recombinant chromosome ($b^+ vg$ or $b\ vg^+$). Fertilization of all

classes of eggs by homozygous recessive sperm ($b\ vg$) produced an offspring population in which 17% exhibited a nonparental, recombinant phenotype, reflecting combinations of alleles not seen before in either P generation parent. In the **Scientific Skills Exercise**, you can use a statistical test to analyze the results from an F_1 dihybrid testcross and see whether the two genes assort independently or are linked.

SCIENTIFIC SKILLS EXERCISE

Using the Chi-Square (χ^2) Test

Are Two Genes Linked or Unlinked? Genes that are in close proximity on the same chromosome will result in the linked alleles being inherited together more often than not. But how can you tell if certain alleles are inherited together due to linkage or whether they just happen to assort together? In this exercise, you will use a simple statistical test, the chi-square (χ^2) test, to analyze phenotypes of F_1 testcross progeny in order to see whether two genes are linked or unlinked.

How These Experiments Are Done If genes are unlinked and assorting independently, the phenotypic ratio of offspring from an F_1 testcross is expected to be 1:1:1:1 (see Figure 15.9). If the two genes are linked, however, the observed phenotypic ratio of the offspring will not match that ratio. Given that random fluctuations in the data do occur, how much must the observed numbers deviate from the expected numbers for us to conclude that the genes are not assorting independently but may instead be linked?

To answer this question, scientists use a statistical test. This test, called a chi-square (χ^2) test, compares an observed data set with an expected data set predicted by a hypothesis (here, that the genes are unlinked) and measures the discrepancy between the two, thus determining the “goodness of fit.” If the discrepancy between the observed and expected data sets is so large that it is unlikely to have occurred by random fluctuation, we say there is statistically significant evidence against the hypothesis (or, more specifically, evidence for the genes being linked). If the discrepancy is small, then our observations are well explained by random variation alone. In this case, we say that the observed data are consistent with our hypothesis, or that the discrepancy is statistically insignificant. Note, however, that consistency with our hypothesis is not the same as proof of our hypothesis. Also, the size of the experimental data set is important: With small data sets like this one, even if the genes are linked, discrepancies might be small by chance alone if the linkage is weak. For simplicity, we overlook the effect of sample size here.

Data from the Simulated Experiment In cosmos plants, purple stem (A) is dominant to green stem (a), and short petals (B) is dominant to long petals (b). In a simulated cross, $AABB$ plants were crossed with $aabb$ plants to generate F_1 dihybrids ($AaBb$), which were then testcrossed ($AaBb \times aabb$). A total of 900 offspring plants were scored for stem color and flower petal length.

Offspring from test-cross of $AaBb (F_1) \times aabb$	Purple stem/short petals ($A-B-$)	Green stem/short petals ($aaB-$)	Purple stem/long petals ($A-bb$)	Green stem/long petals ($aabb$)
Expected ratio if the genes are unlinked	1	1	1	1
Expected number of offspring (of 900)				
Observed number of offspring (of 900)	220	210	231	239

► Cosmos plants



INTERPRET THE DATA

- The results in the data table are from a simulated F_1 dihybrid testcross. The hypothesis that the two genes are unlinked predicts that the offspring phenotypic ratio will be 1:1:1:1. Using this ratio, calculate the expected number of each phenotype out of the 900 total offspring, and enter the values in that data table.
- The goodness of fit is measured by χ^2 . This statistic measures the amounts by which the observed values differ from their respective predictions to indicate how closely the two sets of values match. The formula for calculating this value is

$$\chi^2 = \sum \frac{(o - e)^2}{e}$$

where o = observed and e = expected. Calculate the χ^2 value for the data using the table below. Fill out that table, carrying out the operations indicated in the top row. Then add up the entries in the last column to find the χ^2 value.

Testcross Offspring	Expected (e)	Observed (o)	Deviation (o - e)	(o - e) ²	(o - e) ² /e
($A-B-$)		220			
($aaB-$)		210			
($A-bb$)		231			
($aabb$)		239			
$\chi^2 = \text{Sum}$					

- The χ^2 value means nothing on its own—it is used to find the probability that, assuming the hypothesis is true, the observed data set could have resulted from random fluctuations. A low probability suggests that the observed data are not consistent with the hypothesis and thus the hypothesis should be rejected. A standard cutoff point used by biologists is a probability of 0.05 (5%). If the probability corresponding to the χ^2 value is 0.05 or less, the differences between observed and expected values are considered statistically significant and the hypothesis (that the genes are unlinked) should be rejected. If the probability is above 0.05, the results are not statistically significant; the observed data are consistent with the hypothesis.

To find the probability, locate your χ^2 value in the χ^2 Distribution Table in Appendix F. The “degrees of freedom” (df) of your data set is the number of categories (here, 4 phenotypes) minus 1, so $df = 3$. (a) Determine which values on the $df = 3$ line of the table your calculated χ^2 value lies between. (b) The column headings for these values show the probability range for your χ^2 number. Based on whether there are nonsignificant ($p \leq 0.05$) or significant ($p > 0.05$) differences between the observed and expected values, are the data consistent with the hypothesis that the two genes are unlinked and assorting independently, or is there enough evidence to reject this hypothesis?



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

New Combinations of Alleles: Variation for Natural Selection

EVOLUTION The physical behavior of chromosomes during meiosis contributes to the generation of variation in offspring (see Concept 13.4). Each pair of homologous chromosomes lines up independently of other pairs during metaphase I, and crossing over prior to that, during prophase I, can mix and match parts of maternal and paternal homologs. Mendel's elegant experiments show that the behavior of the abstract entities known as genes—or, more concretely, alleles of genes—also leads to variation in offspring (see Concept 14.1). Now, putting these different ideas together, you can see that the recombinant chromosomes resulting from crossing over may bring alleles together in new combinations, and the subsequent events of meiosis distribute to gametes the recombinant chromosomes in a multitude of combinations, such as the new variants seen in Figures 15.9 and 15.10. Random fertilization then increases even further the number of variant allele combinations that can be created.

This abundance of genetic variation provides the raw material on which natural selection works. If the traits conferred by particular combinations of alleles are better suited for a given environment, organisms possessing those genotypes will be expected to thrive and leave more offspring, ensuring the continuation of their genetic complement. In the next generation, of course, the alleles will be shuffled anew. Ultimately, the interplay between environment and phenotype (and thus genotype) will determine which genetic combinations persist over time.

Mapping the Distance Between Genes Using Recombination Data: *Scientific Inquiry*

The discovery of linked genes and recombination due to crossing over motivated one of Morgan's students, Alfred H. Sturtevant, to work out a method for constructing a **genetic map**, an ordered list of the genetic loci along a particular chromosome.

Sturtevant hypothesized that the percentage of recombinant offspring, the *recombination frequency*, calculated from experiments like the one in Figures 15.9 and 15.10, depends on the distance between genes on a chromosome. He assumed that crossing over is a random event, with the chance of crossing over approximately equal at all points along a chromosome. Based on these assumptions, Sturtevant predicted that *the farther apart two genes are, the higher the probability that a crossover will occur between them and therefore the higher the recombination frequency*. His reasoning was simple: The greater the distance between two genes, the more points there are between them where crossing over can occur. Using recombination data from various fruit fly crosses, Sturtevant proceeded to assign relative positions to genes on the same chromosomes—that is, to *map* genes.

A genetic map based on recombination frequencies is called a **linkage map**. **Figure 15.11** shows Sturtevant's linkage map of three genes: the body color (*b*) and wing size (*vg*) genes depicted in Figure 15.10 and a third gene, called cinnabar (*cn*). Cinnabar is one of many *Drosophila* genes affecting eye color. Cinnabar eyes, a mutant phenotype, are a brighter red than the wild-type color. The recombination frequency between *cn* and *b* is 9%; that between *cn* and *vg*, 9.5%; and that between *b* and *vg*, 17%. In other words, crossovers between *cn* and *b* and between *cn* and *vg* are about half as frequent as crossovers between *b* and *vg*. Only a map that locates *cn* about midway between *b* and *vg* is consistent with these data, as you can prove to yourself by drawing alternative maps. Sturtevant expressed the distances between genes in **map units**, defining one map unit as equivalent to a 1% recombination frequency.

In practice, the interpretation of recombination data is more complicated than this example suggests. Some genes on a chromosome are so far from each other that a crossover between them is virtually certain. The observed frequency of recombination in crosses involving two such genes can have

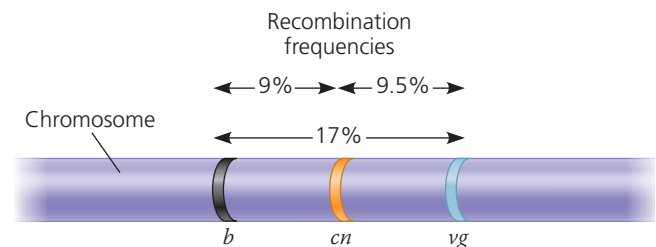
▼ Figure 15.11

Research Method Constructing a Linkage Map

Application A linkage map shows the relative locations of genes along a chromosome.

Technique A linkage map is based on the assumption that the probability of a crossover between two genetic loci is proportional to the distance separating the loci. The recombination frequencies used to construct a linkage map for a particular chromosome are obtained from experimental crosses, such as the cross depicted in Figures 15.9 and 15.10. The distances between genes are expressed as map units, with one map unit equivalent to a 1% recombination frequency. Genes are arranged on the chromosome in the order that best fits the data.

Results In this example, the observed recombination frequencies between three *Drosophila* gene pairs (*b* and *cn*, 9%; *cn* and *vg*, 9.5%; and *b* and *vg*, 17%) best fit a linear order in which *cn* is positioned about halfway between the other two genes:



The *b*-*vg* recombination frequency (17%) is slightly less than the sum of the *b*-*cn* and *cn*-*vg* frequencies ($9 + 9.5 = 18.5\%$) because of the few times that one crossover occurs between *b* and *cn* and another crossover occurs between *cn* and *vg*. The second crossover would "cancel out" the first, reducing the observed *b*-*vg* recombination frequency while contributing to the frequency between each of the closer pairs of genes. The value of 18.5% (18.5 map units) is closer to the actual distance between the genes. In practice, a geneticist would add the smaller distances in constructing a map.

a maximum value of 50%, a result indistinguishable from that for genes on different chromosomes. In this case, the physical connection between genes on the same chromosome is not reflected in the results of genetic crosses. Despite being on the same chromosome and thus being *physically connected*, the genes are *genetically unlinked*; alleles of such genes assort independently, as if they were on different chromosomes. In fact, at least two of the genes for pea characters that Mendel studied are now known to be on the same chromosome, but the distance between them is so great that linkage is not observed in genetic crosses. Consequently, the two genes behaved as if they were on different chromosomes in Mendel's experiments. Genes located far apart on a chromosome are mapped by adding the recombination frequencies from crosses involving closer pairs of genes lying between the two distant genes.

Using recombination data, Sturtevant and his colleagues were able to map numerous *Drosophila* genes in linear arrays. They found that the genes clustered into four groups of linked genes (*linkage groups*). Light microscopy had revealed four pairs of chromosomes in *Drosophila*, so the linkage map provided additional evidence that genes are located on chromosomes. Each chromosome has a linear array of specific genes, each gene with its own locus (**Figure 15.12**).

Because a linkage map is based strictly on recombination frequencies, it gives only an approximate picture of a chromosome. The frequency of crossing over is not actually uniform over the length of a chromosome, as Sturtevant assumed, and therefore map units do not correspond to

actual physical distances (in nanometers, for instance).

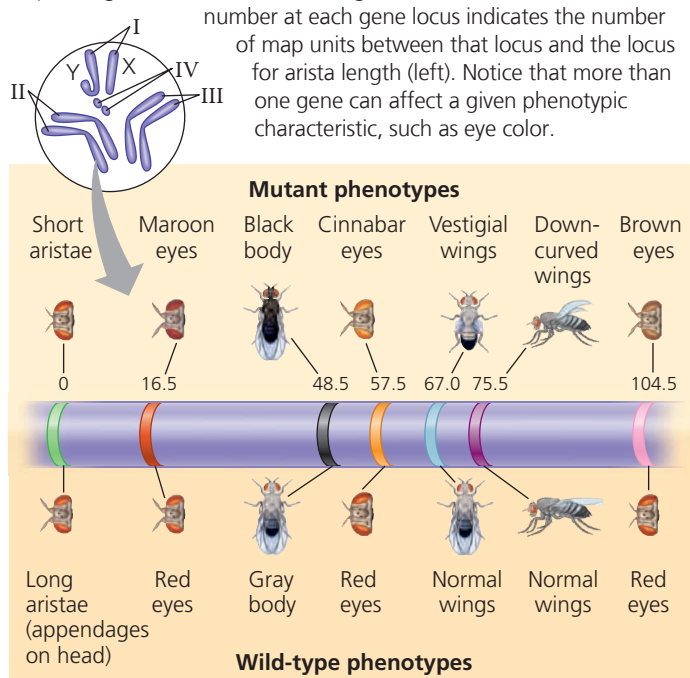
A linkage map does portray the order of genes along a chromosome, but it does not accurately portray the precise locations of those genes. Other methods enable geneticists to construct *cytogenetic maps* of chromosomes, which locate genes with respect to chromosomal features, such as stained bands, that can be seen in the microscope. Technical advances over the last two decades have enormously increased the rate and affordability of DNA sequencing. Therefore, today, most researchers sequence whole genomes to map the locations of genes of a given species. The entire nucleotide sequence is the ultimate physical map of a chromosome, revealing the physical distances in DNA nucleotides between gene loci (see Concept 20.1). Comparing a linkage map with such a physical map or with a cytogenetic map of the same chromosome, we find that the linear order of genes is identical in all the maps, but the spacing between genes is not.

CONCEPT CHECK 15.3

1. When two genes are located on the same chromosome, what is the physical basis for the production of recombinant offspring in a testcross between a dihybrid parent and a double-mutant (recessive) parent?
2. **VISUAL SKILLS** > For each type of offspring of the testcross in Figure 15.9, explain the relationship between its phenotype and the alleles contributed by the female parent. (It will be useful to draw out the chromosomes of each fly and follow the alleles throughout the cross.)
3. **WHAT IF?** > Genes *A*, *B*, and *C* are located on the same chromosome. Testcrosses show that the recombination frequency between *A* and *B* is 28% and that between *A* and *C* is 12%. Can you determine the linear order of these genes? Explain.

For suggested answers, see Appendix A.

▼ Figure 15.12 A partial genetic (linkage) map of a *Drosophila* chromosome. This simplified map shows just seven of the genes that have been mapped on *Drosophila* chromosome II. (DNA sequencing has revealed over 9,000 genes on that chromosome.) The number at each gene locus indicates the number of map units between that locus and the locus for arista length (left). Notice that more than one gene can affect a given phenotypic characteristic, such as eye color.



CONCEPT 15.4

Alterations of chromosome number or structure cause some genetic disorders

As you have learned so far in this chapter, the phenotype of an organism can be affected by small-scale changes involving individual genes. Random mutations are the source of all new alleles, which can lead to new phenotypic traits.

Large-scale chromosomal changes can also affect an organism's phenotype. Physical and chemical disturbances, as well as errors during meiosis, can damage chromosomes in major ways or alter their number in a cell. Large-scale chromosomal alterations in humans and other mammals often lead to spontaneous abortion (miscarriage) of a fetus, and individuals born with these types of genetic defects commonly exhibit various developmental disorders. Plants appear to tolerate such genetic defects better than animals do.

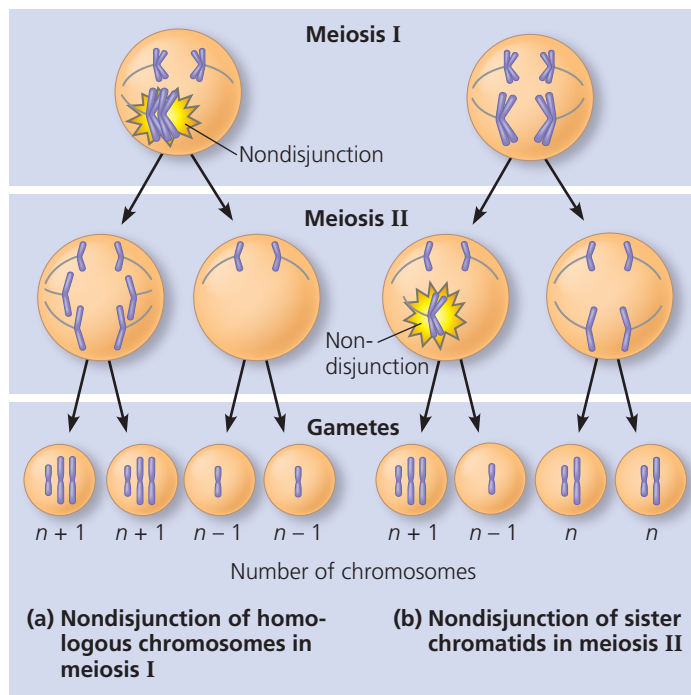
Interview with Terry Orr-Weaver: Why are humans so bad at meiosis?!

Abnormal Chromosome Number

Ideally, the meiotic spindle distributes chromosomes to daughter cells without error. But there is an occasional mishap, called a **nondisjunction**, in which the members of a pair of homologous chromosomes do not move apart properly during meiosis I or sister chromatids fail to separate during meiosis II (**Figure 15.13**). In nondisjunction, one gamete receives two of the same type of chromosome and another gamete receives no copy. The other chromosomes are usually distributed normally.

If either of the aberrant gametes unites with a normal one at fertilization, the zygote will also have an abnormal number of a particular chromosome, a condition known as **aneuploidy**. Fertilization involving a gamete that has no copy of a particular chromosome will lead to a missing chromosome in the zygote (so that the cell has $2n - 1$ chromosomes); the aneuploid zygote is said to be **monosomic** for that chromosome. If a chromosome is present in triplicate in the zygote (so that the cell has $2n + 1$ chromosomes), the aneuploid cell is **trisomic** for that chromosome. Mitosis will subsequently transmit the anomaly to all embryonic cells. Monosomy and trisomy are estimated to occur in 10–25% of human conceptions and are the main reason for pregnancy loss. If the organism survives, it usually has a set of traits caused by the abnormal dose of the genes associated with the extra or missing chromosome. Down syndrome is an example of trisomy in humans that will be

▼ **Figure 15.13 Meiotic nondisjunction.** Gametes with an abnormal chromosome number can arise by nondisjunction in either meiosis I or meiosis II. For simplicity, the figure does not show the spores formed by meiosis in plants. Ultimately, spores form gametes that have the defects shown. (See Figure 13.6.)



discussed later. Nondisjunction can also occur during mitosis. If such an error takes place early in embryonic development, then the aneuploid condition is passed along by mitosis to a large number of cells and is likely to have a substantial effect on the organism.

Some organisms have more than two complete chromosome sets in all somatic cells. The general term for this chromosomal alteration is **polyploidy**; the specific terms *triploidy* ($3n$) and *tetraploidy* ($4n$) indicate three and four chromosomal sets, respectively. One way a triploid cell may arise is by the fertilization of an abnormal diploid egg produced by nondisjunction of all its chromosomes. Tetraploidy could result from the failure of a $2n$ zygote to divide after replicating its chromosomes. Subsequent normal mitotic divisions would then produce a $4n$ embryo.

Polyploidy is fairly common in the plant kingdom. The spontaneous origin of polyploid individuals plays an important role in plant evolution (see Concept 24.2). Many species we eat are polyploid: Bananas are triploid, wheat hexaploid ($6n$), and strawberries octoploid ($8n$). Polyploid animal species are much less common, but there are a few fishes and amphibians known to be polyploid. In general, polyploids are more nearly normal in appearance than aneuploids. One extra (or missing) chromosome apparently disrupts genetic balance more than does an entire extra set of chromosomes.



Alterations of Chromosome Structure

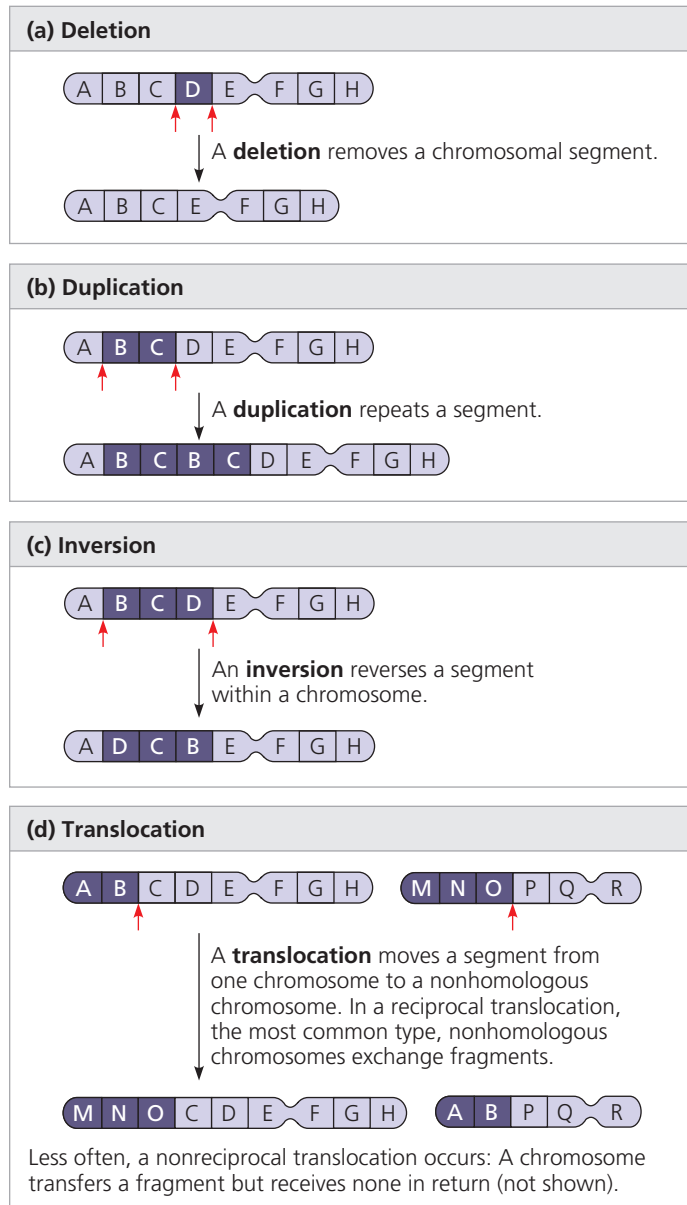
Errors in meiosis or damaging agents such as radiation can cause breakage of a chromosome, which can lead to four types of changes in chromosome structure (**Figure 15.14**).

A **deletion** occurs when a chromosomal fragment is lost. The affected chromosome is then missing certain genes. The “deleted” fragment may become attached as an extra segment to a sister or nonsister chromatid, producing a **duplication** of a portion of that chromosome. A chromosomal fragment may also reattach to the original chromosome but in the reverse orientation, producing an **inversion**. A fourth possible result of chromosomal breakage is for the fragment to join a nonhomologous chromosome, a rearrangement called a **translocation**.

Deletions and duplications are especially likely to occur during meiosis. In crossing over, nonsister chromatids sometimes exchange unequal-sized segments of DNA, so that one partner gives up more genes than it receives (see Figure 20.13). The products of such an unequal crossover are one chromosome with a deletion and one chromosome with a duplication.

A diploid embryo that is homozygous for a large deletion (or has a single X chromosome with a large deletion, in a male) is usually missing a number of essential genes, a condition that is typically lethal. Duplications and translocations also tend to be harmful. In reciprocal translocations, in which segments are exchanged between nonhomologous chromosomes, and in

▼ **Figure 15.14 Alterations of chromosome structure.** Red arrows indicate breakage points. Dark purple highlights the chromosomal parts affected by the rearrangements.



Animation: Alterations of Chromosome Structure

inversions, the balance of genes is not abnormal—all genes are present in their normal doses. Nevertheless, translocations and inversions can alter phenotype because a gene's expression can be influenced by its location among neighboring genes, which can have devastating effects.

Human Disorders Due to Chromosomal Alterations

Alterations of chromosome number and structure are associated with a number of serious human disorders. As described earlier, nondisjunction in meiosis results in aneuploidy in

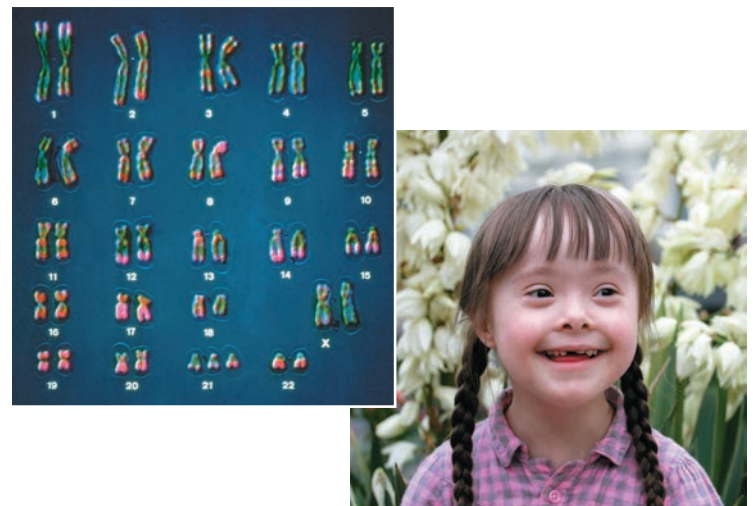
gametes and any resulting zygotes. Although the frequency of aneuploid zygotes may be quite high in humans, most of these chromosomal alterations are so disastrous to development that the affected embryos are spontaneously aborted long before birth. However, some types of aneuploidy appear to upset the genetic balance less than others, so individuals with certain aneuploid conditions can survive to birth and beyond. These individuals have a set of traits—a *syndrome*—characteristic of the type of aneuploidy. Genetic disorders caused by aneuploidy can be diagnosed before birth by fetal testing (see Figure 14.19).

Down Syndrome (Trisomy 21)

One aneuploid condition, **Down syndrome**, affects approximately one out of every 830 children born in the United States (**Figure 15.15**). Down syndrome is usually the result of an extra chromosome 21, so that each body cell has a total of 47 chromosomes. Because the cells are trisomic for chromosome 21, Down syndrome is often called *trisomy 21*. Down syndrome includes characteristic facial features, short stature, correctable heart defects, and developmental delays. Individuals with Down syndrome have an increased chance of developing leukemia and Alzheimer's disease but have a lower rate of high blood pressure, atherosclerosis (hardening of the arteries), stroke, and many types of solid tumors. Although people with Down syndrome, on average, have a life span shorter than normal, most, with proper medical treatment, live to middle age and beyond. Many live independently or at home with their families, are employed, and are valuable contributors to their communities. Almost all males and about half of females with Down syndrome are sexually underdeveloped and sterile.

The frequency of Down syndrome increases with the age of the mother. While the disorder occurs in just 0.04% (4 out

▼ **Figure 15.15 Down syndrome.** The karyotype shows trisomy 21, the most common cause of Down syndrome. The child exhibits the facial features characteristic of this disorder.



of 10,000 births) of children born to women under age 30, the risk climbs to 0.92% (92 out of 10,000) for mothers at age 40 and is even higher for older mothers. The correlation of Down syndrome with maternal age has not yet been explained. Most cases result from nondisjunction during meiosis I, and some research points to an age-dependent abnormality in meiosis. Trisomies of some other chromosomes also increase in incidence with maternal age, although infants with other autosomal trisomies rarely survive for long. Medical experts recommend that prenatal screening for trisomies in the embryo be offered to all pregnant women, due to its low risk and useful results. In 2008, a law was passed stipulating that medical practitioners give accurate, up-to-date information about any prenatal or postnatal diagnosis received by parents and that they connect parents with appropriate support services.

Aneuploidy of Sex Chromosomes

Aneuploid conditions involving sex chromosomes appear to upset the genetic balance less than those involving autosomes. This may be because the Y chromosome carries relatively few genes. Also, extra copies of the X chromosome simply become inactivated as Barr bodies.

An extra X chromosome in a male, producing XXY, occurs approximately once in every 500 to 1,000 live male births. People with this disorder, called *Klinefelter syndrome*, have male sex organs, but the testes are abnormally small and the man is sterile. Even though the extra X is inactivated, some breast enlargement and other female body characteristics are common. Affected individuals may have subnormal intelligence. About 1 of every 1,000 males is born with an extra Y chromosome (XYY). These males undergo normal sexual development and do not exhibit any well-defined syndrome, but tend to be taller than average.

Females with trisomy X (XXX), which occurs once in approximately 1,000 live female births, are healthy and have no unusual physical features other than being slightly taller than average. Triple-X females are at risk for learning disabilities but are fertile. Monosomy X, which is called *Turner syndrome*, occurs about once in every 2,500 female births and is the only known viable monosomy in humans. Although these XO individuals are phenotypically female, they are sterile because their sex organs do not mature. When provided with estrogen replacement therapy, girls with Turner syndrome do develop secondary sex characteristics. Most have normal intelligence.

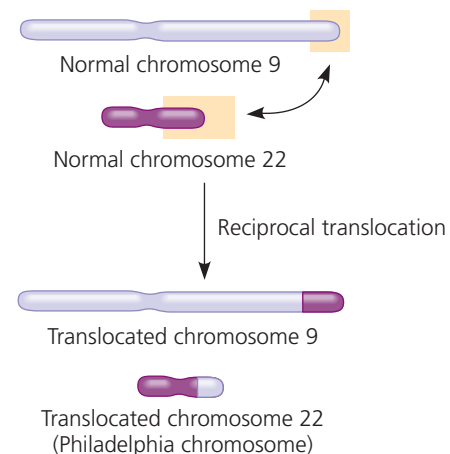
Disorders Caused by Structurally Altered Chromosomes

Many deletions in human chromosomes, even in a heterozygous state, cause severe problems. One such syndrome, known as *cri du chat* (“cry of the cat”), results from a specific deletion in chromosome 5. A child born with this deletion is severely intellectually disabled, has a small head with unusual

facial features, and has a cry that sounds like the mewing of a distressed cat. Such individuals usually die in infancy or early childhood.

Chromosomal translocations can also occur during mitosis; some have been implicated in certain cancers, including *chronic myelogenous leukemia (CML)*. This disease occurs when a reciprocal translocation happens during mitosis of cells that are precursors of white blood cells. The exchange of a large portion of chromosome 22 with a small fragment from a tip of chromosome 9 produces a much shortened, easily recognized chromosome 22, called the *Philadelphia chromosome* (Figure 15.16). Such an exchange causes cancer by creating a new “fused” gene that leads to uncontrolled cell cycle progression. (The mechanism of gene activation will be discussed in Chapter 18.)

✓ Figure 15.16 Translocation associated with chronic myelogenous leukemia (CML). The cancerous cells in nearly all CML patients contain an abnormally short chromosome 22, the so-called Philadelphia chromosome, and an abnormally long chromosome 9. These altered chromosomes result from the reciprocal translocation shown here, which presumably occurred in a single white blood cell precursor undergoing mitosis and was then passed along to all descendant cells.



CONCEPT CHECK 15.4

1. About 5% of individuals with Down syndrome have a chromosomal translocation in which a third copy of chromosome 21 is attached to chromosome 14. If this translocation occurred in a parent's gonad, how could it lead to Down syndrome in a child?
2. **MAKE CONNECTIONS** ▶ The ABO blood type locus has been mapped on chromosome 9. A father who has type AB blood and a mother who has type O blood have a child with trisomy 9 and type A blood. Using this information, can you tell in which parent the nondisjunction occurred? Explain your answer. (See Figures 14.11 and 15.13.)
3. **MAKE CONNECTIONS** ▶ The gene that is activated on the Philadelphia chromosome codes for an intracellular tyrosine kinase. Review the discussion of cell cycle control in Concept 12.3, and explain how the activation of this gene could contribute to the development of cancer.

For suggested answers, see Appendix A.

CONCEPT 15.5

Some inheritance patterns are exceptions to standard Mendelian inheritance

In the previous section, you learned about deviations from the usual patterns of chromosomal inheritance due to abnormal events in meiosis and mitosis. We conclude this chapter by describing two normally occurring exceptions to Mendelian genetics, one involving genes located in the nucleus and the other involving genes located outside the nucleus. In both cases, the sex of the parent contributing an allele is a factor in the pattern of inheritance.

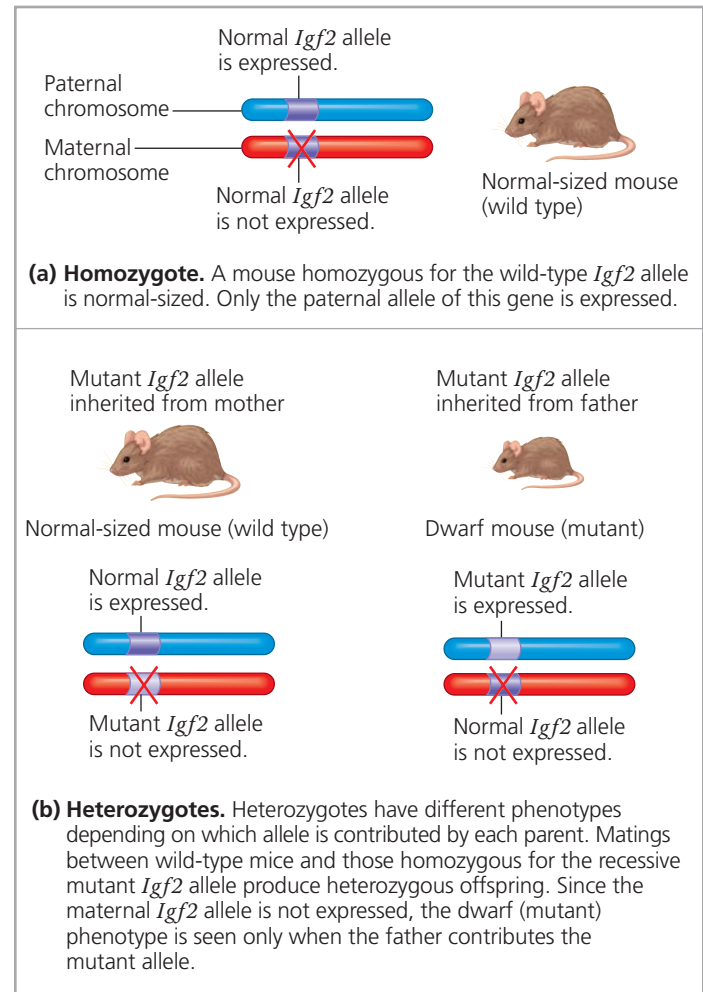
Genomic Imprinting

Throughout our discussions of Mendelian genetics and the chromosomal basis of inheritance, we have assumed that a given allele will have the same effect whether it was inherited from the mother or the father. This is probably a safe assumption most of the time. For example, when Mendel crossed purple-flowered pea plants with white-flowered pea plants, he observed the same results regardless of whether the purple-flowered parent supplied the eggs or the sperm. In recent years, however, geneticists have identified a number of traits in mammals that depend on which parent passed along the alleles for those traits. Such variation in phenotype depending on whether an allele is inherited from the male or female parent is called **genomic imprinting**. (Note that unlike sex-linked genes, most imprinted genes are on autosomes.) Using newer DNA sequence-based methods, about 100 imprinted genes have been identified in humans, and 125 in mice.

Genomic imprinting occurs during gamete formation and results in the silencing of a particular allele of certain genes. Because these genes are imprinted differently in sperm and eggs, the offspring expresses only one allele of an imprinted gene, the one that has been inherited from a specific parent—either the female parent or the male parent, depending on the particular gene. The imprints are then transmitted to all body cells during development. In each generation, the old imprints are “erased” in gamete-producing cells, and the chromosomes of the developing gametes are newly imprinted according to the sex of the individual forming the gametes. In a given species, the imprinted genes are always imprinted in the same way. For instance, a gene imprinted for maternal allele expression is always imprinted this way, generation after generation.

Consider, for example, the mouse gene for insulin-like growth factor 2 (*Igf2*), one of the first imprinted genes to be identified. Although this growth factor is required for normal prenatal growth, only the paternal allele is expressed (**Figure 15.17a**). Evidence that the *Igf2* gene is imprinted came initially from crosses between normal-sized

✓ **Figure 15.17** Genomic imprinting of the mouse *Igf2* gene.



(wild-type) mice and dwarf (mutant) mice homozygous for a recessive mutation in the *Igf2* gene. The phenotypes of heterozygous offspring (with one normal allele and one mutant) differed depending on whether the mutant allele came from the mother or the father (**Figure 15.17b**).

What exactly is a genomic imprint? It turns out that imprinting can involve either silencing an allele in one type of gamete (egg or sperm) or activating it in the other. In many cases, the imprint seems to consist of methyl ($-\text{CH}_3$) groups that are added to cytosine nucleotides of one of the alleles. Such methylation may silence the allele, an effect consistent with evidence that heavily methylated genes are usually inactive (see Concept 18.2). However, for a few genes, methylation has been shown to *activate* expression of the allele. This is the case for the *Igf2* gene: Methylation of certain cytosines on the paternal chromosome leads to expression of the paternal *Igf2* allele, by an indirect mechanism involving chromatin structure and protein-DNA interactions.

Genomic imprinting may affect only a small fraction of the genes in mammalian genomes, but most of the known imprinted genes are critical for embryonic development. In experiments with mice, embryos engineered to inherit both copies of certain chromosomes from the same parent usually

die before birth, whether that parent is male or female. A few years ago, however, scientists in Japan combined the genetic material from two eggs in a zygote while allowing expression of the *Igf2* gene, along with several other imprinted genes, from only one of the egg nuclei. The zygote developed into an apparently healthy mouse. Normal development seems to require that embryonic cells have exactly one active copy—not zero, not two—of certain genes. The association of improper imprinting with abnormal development and certain cancers has stimulated ongoing studies of how different genes are imprinted.



Interview with Shirley Tilghman: Discovering imprinted genes

Inheritance of Organelle Genes

Although our focus in this chapter has been on the chromosomal basis of inheritance, we end with an important amendment: Not all of a eukaryotic cell's genes are located on nuclear chromosomes, or even in the nucleus; some genes are located in organelles in the cytoplasm. Because they are outside the nucleus, these genes are sometimes called *extranuclear genes* or *cytoplasmic genes*. Mitochondria, as well as chloroplasts and other plastids in plants, contain small circular DNA molecules that carry a number of genes. These organelles reproduce themselves and transmit their genes to daughter organelles. Genes on organelle DNA are not distributed to offspring according to the same rules that direct the distribution of nuclear chromosomes during meiosis, so they do not display Mendelian inheritance.

The first hint that extranuclear genes exist came from studies by the German scientist Carl Correns on the inheritance of yellow or white patches on the leaves of an otherwise green plant. In 1909, he observed that the coloration of the offspring was determined only by the maternal parent (the source of eggs) and not by the paternal parent (the source of sperm). Subsequent research showed that such coloration patterns, or variegation, are due to mutations in plastid genes that control pigmentation (**Figure 15.18**). In most plants, a zygote receives all its plastids from the cytoplasm of the egg and none from the sperm, which contributes little more than a haploid set of chromosomes. An egg may contain plastids with different alleles for a pigmentation gene. As the zygote develops, plastids containing wild-type or mutant pigmentation genes are distributed randomly to daughter cells. The pattern of leaf coloration exhibited by a plant depends on the ratio of wild-type to mutant plastids in its various tissues.

Similar maternal inheritance is also the rule for mitochondrial genes in most animals and plants, because



◀ Figure 15.18 A painted nettle coleus plant. The variegated (patterned) leaves on this coleus plant (*Plectranthus scutellarioides*) result from mutations that affect expression of pigment genes located in plastids, which generally are inherited from the maternal parent.

the mitochondria passed on to a zygote come from the cytoplasm of the egg. (The few mitochondria contributed by the sperm appear to be destroyed in the egg by autophagy; see Figure 7.13.) The products of most mitochondrial genes help make up some of the protein complexes of the electron transport chain and ATP synthase (see Figure 10.15). Defects in one or more of these proteins, therefore, reduce the amount of ATP the cell can make and have been shown to cause a number of human disorders in as many as one out of every 5,000 births. Because the parts of the body most susceptible to energy deprivation are the nervous system and the muscles, most mitochondrial diseases primarily affect these systems. For example, *mitochondrial myopathy* causes weakness, intolerance of exercise, and muscle deterioration. Another mitochondrial disorder is *Leber's hereditary optic neuropathy*, which can produce sudden blindness in people as young as their 20s or 30s. The four mutations found thus far to cause this disorder affect oxidative phosphorylation during cellular respiration, a crucial function for the cell (see Concept 10.4).

The fact that mitochondrial disorders are inherited only from the mother suggests a way to avoid passing along these disorders. The chromosomes from the egg of an affected mother could be transferred to an egg of a healthy donor that has had its own chromosomes removed. This “two-mother” egg could then be fertilized by a sperm from the prospective father and transplanted into the womb of the prospective mother, becoming an embryo with three parents. After optimizing conditions for this approach in monkeys, researchers reported in 2013 that they have successfully carried out this procedure on human eggs. More research will be necessary to optimize experimental conditions for the health of the embryo, and eventual use of this procedure would require approval by the relevant federal agencies.

In addition to the rarer diseases clearly caused by defects in mitochondrial DNA, mitochondrial mutations inherited from a person's mother may contribute to at least some types of diabetes and heart disease, as well as to other disorders that commonly debilitate the elderly, such as Alzheimer's disease. In the course of a lifetime, new mutations gradually accumulate in our mitochondrial DNA, and some researchers think that these mutations play a role in the normal aging process.

CONCEPT CHECK 15.5

1. Gene dosage—the number of copies of a gene that are actively being expressed—is important to proper development. Identify and describe two processes that establish the proper dosage of certain genes.
2. Reciprocal crosses between two primrose varieties, A and B, produced the following results: A female $\times$ B male $\rightarrow$ offspring with all green (nonvariegated) leaves; B female $\times$ A male $\rightarrow$ offspring with patterned (variegated) leaves. Explain these results.
3. **WHAT IF? ▶** Mitochondrial genes are critical to the energy metabolism of cells, but mitochondrial disorders caused by mutations in these genes are generally not lethal. Why not?

For suggested answers, see Appendix A.

15 Chapter Review



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SUMMARY OF KEY CONCEPTS

CONCEPT 15.1

Morgan showed that Mendelian inheritance has its physical basis in the behavior of chromosomes: scientific inquiry (pp. 346–347)



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax

- Morgan's work with an eye color gene in *Drosophila* led to the **chromosome theory of inheritance**, which states that genes are located on chromosomes and that the behavior of chromosomes during meiosis accounts for Mendel's laws.

? What characteristic of the sex chromosomes allowed Morgan to correlate their behavior with that of the alleles of the eye color gene?

CONCEPT 15.2

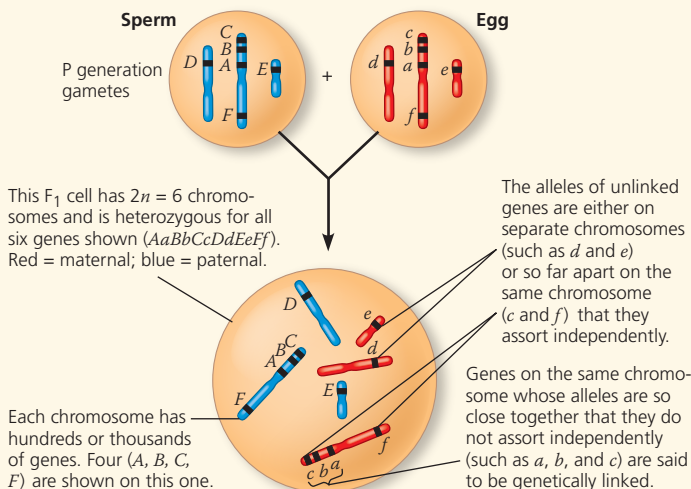
Sex-linked genes exhibit unique patterns of inheritance (pp. 348–350)

- Sex is often chromosomally based. Humans and other mammals have an X-Y system in which sex is largely determined by whether a Y chromosome is present. Other systems are found in birds, fishes, and insects.
- The sex chromosomes carry **sex-linked genes**, virtually all of which are on the X chromosome (**X-linked**). Any male who inherits a recessive X-linked allele (from his mother) will express the trait, such as color blindness.
- In mammalian females, one of the two X chromosomes in each cell is randomly inactivated during early embryonic development, becoming highly condensed into a **Barr body**.

? Why are males affected by X-linked disorders much more often than females?

CONCEPT 15.3

Linked genes tend to be inherited together because they are located near each other on the same chromosome (pp. 351–356)



- An F₁ dihybrid testcross yields **parental types** with the same combination of traits as those in the P generation parents and **recombinant types (recombinants)** with new combinations of traits not seen in either P generation parent. Because of the independent assortment of chromosomes, unlinked genes exhibit a 50% frequency of recombination in the gametes. For genetically **linked genes**, **crossing over** between nonsister chromatids during meiosis I accounts for the observed recombinants, always less than 50%.
- The order of genes on a chromosome and the relative distances between them can be deduced from recombination frequencies observed in genetic crosses. These data allow construction of a **linkage map** (a type of **genetic map**). The farther apart genes are, the more likely their allele combinations will be recombined during crossing over.

? Why are specific alleles of two distant genes more likely to show recombination than those of two closer genes?

CONCEPT 15.4

Alterations of chromosome number or structure cause some genetic disorders (pp. 356–359)

- Aneuploidy**, an abnormal chromosome number, can result from **nondisjunction** during meiosis. When a normal gamete unites with one containing two copies or no copies of a particular chromosome, the resulting zygote and its descendant cells either have one extra copy of that chromosome (**trisomy**, 2n + 1) or are missing a copy (**monosomy**, 2n - 1). **Polyploidy** (extra sets of chromosomes) can result from complete nondisjunction.
- Chromosome breakage can result in alterations of chromosome structure: **deletions**, **duplications**, **inversions**, and **translocations**. Translocations can be reciprocal or nonreciprocal.
- Changes in the number of chromosomes per cell or in the structure of individual chromosomes can affect the phenotype and, in some cases, lead to disorders. Such alterations cause **Down syndrome** (usually due to trisomy of chromosome 21), certain cancers associated with chromosomal translocations that occur during mitosis, and various other human disorders.

? Why are inversions and reciprocal translocations less likely to be lethal than are aneuploidy, duplications, deletions, and nonreciprocal translocations?

CONCEPT 15.5

Some inheritance patterns are exceptions to standard Mendelian inheritance (pp. 360–361)

- In mammals, the phenotypic effects of a small number of particular genes depend on which allele is inherited from each parent, a phenomenon called **genomic imprinting**. Imprints are formed during gamete production, with the result that one allele (either maternal or paternal) is not expressed in offspring.
- The inheritance of traits controlled by the genes present in mitochondria and plastids depends solely on the maternal parent because the zygote's cytoplasm containing these organelles comes from the egg. Some diseases affecting the nervous and muscular systems are caused by defects in mitochondrial genes that prevent cells from making enough ATP.

? Explain how genomic imprinting and inheritance of mitochondrial and chloroplast DNA are exceptions to standard Mendelian inheritance.

TEST YOUR UNDERSTANDING

1. A man with hemophilia (a recessive, sex-linked condition) has a daughter without the condition. She marries a man who does not have hemophilia. What is the probability that their daughter will have hemophilia? Their son? If they have four sons, what is the probability that all will be affected?
2. Polydactyly is a condition in which an individual is born with extra fingers or toes. It is an autosomal dominant trait when it occurs in the absence of any other congenital abnormality. Peter's parents and children have polydactyly, but he has the normal number of digits. What may have happened in Peter's case?
3. A wild-type fruit fly (heterozygous for gray body color and normal wings) is mated with a black fly with vestigial wings. The offspring have the following phenotypic distribution: wild-type, 778; black vestigial, 785; black normal, 158; gray vestigial, 162. What is the recombination frequency between these genes for body color and wing size? Is this consistent with the results of the experiment in Figure 15.9?
4. A planet is inhabited by creatures that reproduce with the same hereditary patterns seen in humans. Three phenotypic characters are height (T = tall, t = dwarf), head appendages (A = antennae, a = no antennae), and nose morphology (S = upturned snout, s = downturned snout). Since the creatures are not "intelligent," Earth scientists are able to do some controlled breeding experiments using various heterozygotes in testcrosses. For tall heterozygotes with antennae, the offspring are tall antennae, 46; dwarf antennae, 7; dwarf no antennae, 42; tall no antennae, 5. For heterozygotes with antennae and an upturned snout, the offspring are antennae upturned snout, 47; antennae downturned snout, 2; no antennae downturned snout, 48; no antennae upturned snout, 3. Calculate the recombination frequencies for both experiments.
5. Using the information from problem 4, scientists do a further testcross using a heterozygote for height and nose morphology. The offspring are tall upturned snout, 40; dwarf upturned snout, 9; dwarf downturned snout, 42; tall downturned snout, 9. Calculate the recombination frequency from these data, and then use your answer from problem 4 to determine the correct order of the three linked genes.
6. A wild-type fruit fly (heterozygous for gray body color and red eyes) is mated with a black fruit fly with purple eyes. The offspring are wild-type, 721; black purple, 751; gray purple, 49; black red, 45. What is the recombination frequency between these genes for body color and eye color? Using information from problem 3, what fruit flies (genotypes and phenotypes) would you mate to determine the order of the body color, wing size, and eye color genes on the chromosome?
7. Assume that genes A and B are on the same chromosome and are 50 map units apart. An animal heterozygous at both loci is crossed with one that is homozygous recessive at both loci. What percentage of the offspring will show recombinant phenotypes resulting from crossovers? Without knowing these genes are on the same chromosome, how would you interpret the results of this cross?
8. Two genes of a flower, one controlling blue (B) versus white (b) petals and the other controlling round (R) versus oval (r) stamens, are linked and are 10 map units apart. You cross a homozygous blue oval plant with a homozygous white round plant. The resulting F_1 progeny are crossed with homozygous



PRACTICE
TEST
goo.gl/iAsVgI

white oval plants, and 1,000 offspring plants are obtained. How many plants of each of the four phenotypes do you expect?

9. A *Drosophila* with long wings and red eyes is mated with one that has short wings and white eyes. Out of the 200 offspring obtained, 49 have long wings and red eyes, 52 have long wings and white eyes, 48 have short wings and red eyes, and 51 have short wings and white eyes. Are the genes encoding wing size and eye color linked? Explain.
10. Banana plants, which are triploid, are seedless and therefore sterile. Propose a possible explanation.
11. **EVOLUTION CONNECTION** Crossing over is thought to be evolutionarily advantageous because it continually shuffles genetic alleles into novel combinations. Until recently, it was thought that the genes on the Y chromosome might degenerate because they lack homologous genes on the X chromosome with which to pair up prior to crossing over. However, when the Y chromosome was sequenced, eight large regions were found to be internally homologous to each other, and quite a few of the 78 genes represent duplicates. (Y chromosome researcher David Page has called it a "hall of mirrors.") Explain what might be a benefit of these regions.
12. **SCIENTIFIC INQUIRY • DRAW IT** Assume you are mapping genes A , B , C , and D in *Drosophila*. You know that these genes are linked on the same chromosome, and you determine the recombination frequencies between each pair of genes to be as follows: $A-B$, 8%; $A-C$, 28%; $A-D$, 25%; $B-C$, 20%; $B-D$, 33%.
(a) Describe how you determined the recombination frequency for each pair of genes.
(b) Draw a chromosome map based on your data.
13. **WRITE ABOUT A THEME: INFORMATION** The continuity of life is based on heritable information in the form of DNA. In a short essay (100–150 words), relate the structure and behavior of chromosomes to inheritance in both asexually and sexually reproducing species.
14. **SYNTHESIZE YOUR KNOWLEDGE**

Butterflies have an X-Y sex determination system that is different from that of flies or humans. Female butterflies may be either XY or XO, while butterflies with two or more X chromosomes are males. This photograph shows a tiger swallowtail *gynandromorph*, which is half male (left side) and half female (right side). Given that the first division of the zygote divides the embryo into the future right and left halves of the butterfly, propose a hypothesis that explains how nondisjunction during the first mitosis might have produced this unusual-looking butterfly.



For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Nucleic Acids and Inheritance

16

▲ **Figure 16.1** What is the structure of DNA?

KEY CONCEPTS

- 16.1** DNA is the genetic material
- 16.2** Many proteins work together in DNA replication and repair
- 16.3** A chromosome consists of a DNA molecule packed together with proteins



▲ James Watson (left) and Francis Crick with their DNA model.

Life's Operating Instructions

The elegant double-helical structure of deoxyribonucleic acid, or DNA, has become an icon of modern biology (**Figure 16.1**). James Watson and Francis Crick shook the scientific world in April 1953 with their DNA model, which they constructed from sheet metal and wire, shown in the small photo. Gregor Mendel's heritable factors and Thomas Hunt Morgan's genes on chromosomes are, in fact, composed of DNA. Chemically speaking, your genetic endowment is the DNA you inherited from your parents. DNA, the substance of inheritance, is the most celebrated molecule of our time.

Of all nature's molecules, nucleic acids are unique in their ability to direct their own replication from monomers. Indeed, the resemblance of offspring to their parents has its basis in the accurate replication of DNA and its transmission from one generation to the next. Hereditary information in DNA directs the development of your biochemical, anatomical, physiological, and, to some extent, behavioral traits. In this chapter, you will discover how biologists deduced that DNA is the genetic material and how Watson and Crick worked out its structure. You will also learn how a molecule of DNA is copied during **DNA replication** and how cells repair their DNA. Finally, you will explore how a molecule of DNA is packaged together with proteins in a chromosome.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter



Interview with James Watson: Co-discoverer of the structure of DNA

CONCEPT 16.1

DNA is the genetic material

Today, even schoolchildren have heard of DNA, and scientists routinely manipulate DNA in the laboratory. Early in the 20th century, however, identifying the molecules of inheritance loomed as a major challenge to biologists.

The Search for the Genetic Material: Scientific Inquiry

Once T. H. Morgan's group showed that genes exist as parts of chromosomes (described in Concept 15.1), the two chemical components of chromosomes—DNA and protein—emerged as the leading candidates for the genetic material. Until the 1940s, the case for proteins seemed stronger: Biochemists had identified proteins as a class of macromolecules with great heterogeneity and specificity of function, essential requirements for the hereditary material. Moreover, little was known about nucleic acids, whose physical and chemical properties seemed far too uniform to account for the multitude of specific inherited traits exhibited by every organism. This view gradually changed as the role of DNA in heredity was worked out in studies of bacteria and the viruses that infect them, systems far simpler than fruit flies or humans. Let's trace the search for the genetic material as a case study in scientific inquiry.

Evidence That DNA Can Transform Bacteria

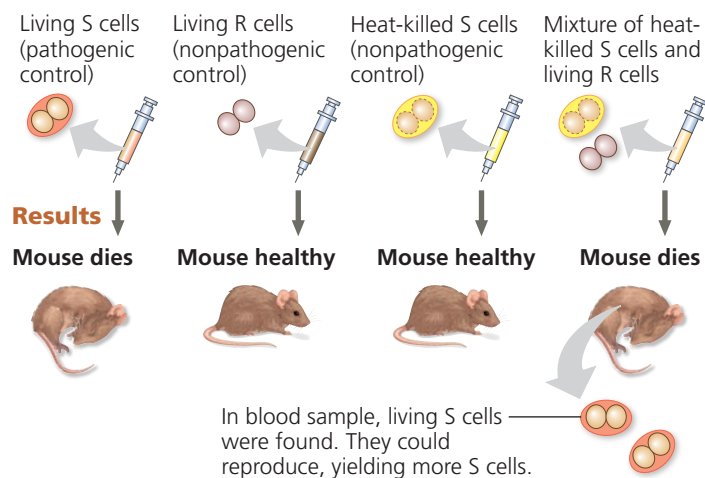
In 1928, a British medical officer named Frederick Griffith was trying to develop a vaccine against pneumonia. He was studying *Streptococcus pneumoniae*, a bacterium that causes pneumonia in mammals. Griffith had two strains (varieties) of the bacterium, one pathogenic (disease-causing) and one nonpathogenic (harmless). He was surprised to find that when he killed the pathogenic bacteria with heat and then mixed the cell remains with living bacteria of the nonpathogenic strain, some of the living cells became pathogenic (Figure 16.2). Furthermore, this newly acquired trait of pathogenicity was inherited by all the descendants of the transformed bacteria. Apparently, some chemical component of the dead pathogenic cells caused this heritable change, although the identity of the substance was not known. Griffith called the phenomenon **transformation**, now defined as a change in genotype and phenotype due to the assimilation of external DNA by a cell. Later work by Oswald Avery, Maclyn McCarty, and Colin MacLeod identified the transforming substance as DNA.

Scientists remained skeptical, however, since many still viewed proteins as better candidates for the genetic material. Also, many biologists were not convinced that bacterial genes would be similar in composition and function to those of more complex organisms. But the major reason for the continued doubt was that so little was known about DNA.

Figure 16.2

Inquiry Can a genetic trait be transferred between different bacterial strains?

Experiment Frederick Griffith studied two strains of the bacterium *Streptococcus pneumoniae*. The S (smooth) strain can cause pneumonia in mice; it is pathogenic because the cells have an outer capsule that protects them from an animal's immune system. Cells of the R (rough) strain lack a capsule and are nonpathogenic. To test for the trait of pathogenicity, Griffith injected mice with the two strains:



Conclusion The living R bacteria had been transformed into pathogenic S bacteria by an unknown, heritable substance from the dead S cells that enabled the R cells to make capsules.

Data from F. Griffith, The significance of pneumococcal types, *Journal of Hygiene* 27:113–159 (1928).

WHAT IF? > How did this experiment rule out the possibility that the R cells simply used the dead S cells' capsules to become pathogenic?

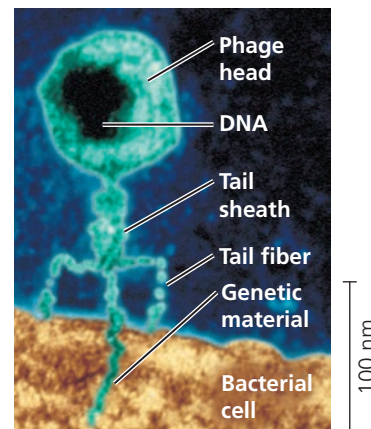
Evidence That Viral DNA Can Program Cells

Additional evidence that DNA was the genetic material came from studies of viruses that infect bacteria (Figure 16.3). These viruses are called **bacteriophages** (meaning "bacteria-eaters"), or **phages** for short. Viruses are much simpler than cells. A **virus** is little more than DNA (or sometimes RNA) enclosed by a protective coat, which is often simply protein. To produce more viruses, a virus must infect a cell and take over the cell's metabolic machinery.

Phages have been widely used as tools by researchers in molecular genetics. In 1952, Alfred Hershey and Martha Chase performed

> Figure 16.3 A virus infecting a bacterial cell.

A phage called T2 attaches to a host cell and injects its genetic material through the plasma membrane, while the head and tail parts remain on the outer bacterial surface (colorized TEM).



experiments showing that DNA is the genetic material of a phage known as T2. This is one of many phages that infect *Escherichia coli* (*E. coli*), a bacterium that normally lives in the intestines of mammals and is a model organism for molecular biologists. At that time, biologists already knew that T2, like many other phages, was composed almost entirely of DNA and protein. They also knew that the T2 phage could quickly turn an *E. coli* cell into a T2-producing factory that released many copies of new phages when the cell ruptured. Somehow, T2 could reprogram its host cell to produce viruses. But which viral component—protein or DNA—was responsible?

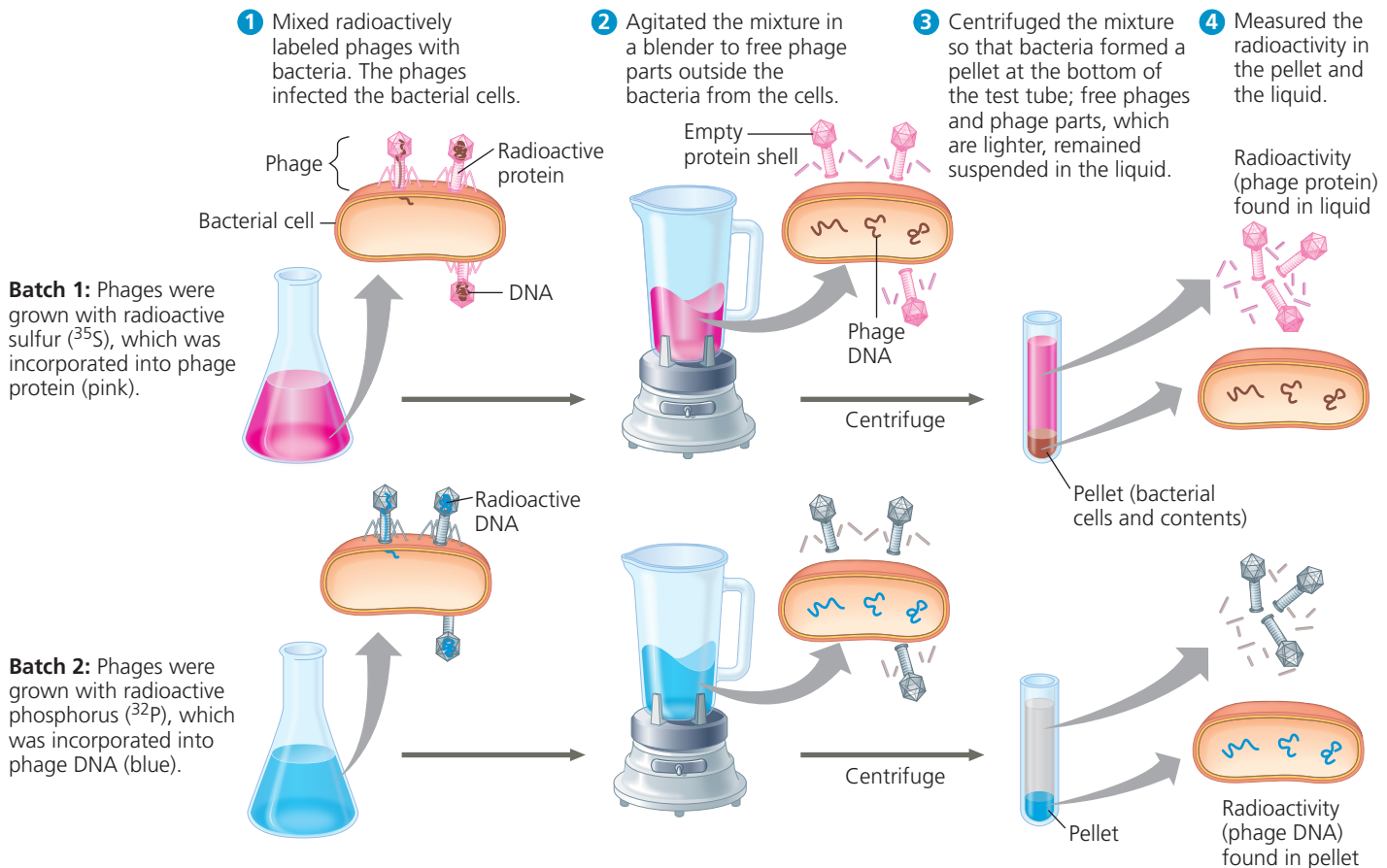
Hershey and Chase answered this question by devising an experiment showing that only one of the two components of

T2 actually enters the *E. coli* cell during infection (**Figure 16.4**). In their experiment, they used a radioactive isotope of sulfur to tag protein in one batch of T2 and a radioactive isotope of phosphorus to tag DNA in a second batch. Because protein, but not DNA, contains sulfur, radioactive sulfur atoms were incorporated only into the protein of the phage. In a similar way, the atoms of radioactive phosphorus labeled only the DNA, not the protein, because nearly all the phage's phosphorus is in its DNA. In the experiment, separate samples of nonradioactive *E. coli* cells were infected with the protein-labeled and DNA-labeled batches of T2. The researchers then tested the two samples shortly after the onset of infection to see which type of molecule—protein or DNA—had entered the bacterial cells and would therefore be capable of reprogramming them.

▼ Figure 16.4

Inquiry Is protein or DNA the genetic material of phage T2?

Experiment Alfred Hershey and Martha Chase used radioactive sulfur and phosphorus to trace the fates of protein and DNA, respectively, of T2 phages that infected bacterial cells. They wanted to see which of these molecules entered the cells and could reprogram them to make more phages.



Results When proteins were labeled (batch 1), radioactivity remained outside the cells, but when DNA was labeled (batch 2), radioactivity was found inside the cells. Cells containing radioactive phage DNA released new phages with some radioactive phosphorus.

Conclusion Phage DNA entered bacterial cells, but phage proteins did not. Hershey and Chase concluded that DNA, not protein, functions as the genetic material of phage T2.

Data from A. D. Hershey and M. Chase, Independent functions of viral protein and nucleic acid in growth of bacteriophage, *Journal of General Physiology* 36:39–56 (1952).

WHAT IF? > How would the results have differed if proteins carried the genetic information?



Animation: The Hershey-Chase Experiment

Hershey and Chase found that the phage DNA entered the host cells but the phage protein did not. Moreover, when these bacteria were returned to a culture medium and the infection ran its course, the *E. coli* released phages that contained some radioactive phosphorus. This result further showed that the DNA inside the cell played an ongoing role during the infection process. They concluded that the DNA injected by the phage must be the molecule carrying the genetic information that makes the cells produce new viral DNA and proteins. The Hershey-Chase experiment was a landmark study because it provided powerful evidence that nucleic acids, rather than proteins, are the hereditary material, at least for certain viruses.

Additional Evidence That DNA Is the Genetic Material

Further evidence that DNA is the genetic material came from the laboratory of biochemist Erwin Chargaff. DNA was known to be a polymer of nucleotides, each having three components: a nitrogenous (nitrogen-containing) base, a pentose sugar called deoxyribose, and a phosphate group (**Figure 16.5**). The base can be adenine (A), thymine (T), guanine (G), or cytosine (C). Chargaff analyzed the base composition of DNA from a number of different organisms. In 1950, he reported that the base composition of DNA varies from one species to another. For example, he found that 32.8% of sea urchin DNA nucleotides have the base A, whereas 30.4% of human DNA nucleotides have the base A and only 24.7% of the DNA nucleotides from the bacterium *E. coli* have the base A. Chargaff's evidence of molecular diversity among species, which most scientists had presumed to be absent from DNA, made DNA a more credible candidate for the genetic material.

Chargaff also noticed a peculiar regularity in the ratios of nucleotide bases. In the DNA of each species he studied, the number of adenines approximately equaled the number of thymines, and the number of guanines approximately equaled the number of cytosines. In sea urchin DNA, for example, Chargaff's analysis found the four bases in these percentages: A = 32.8% and T = 32.1%; G = 17.7% and C = 17.3%. (The percentages are not exactly the same because of limitations in Chargaff's techniques.)

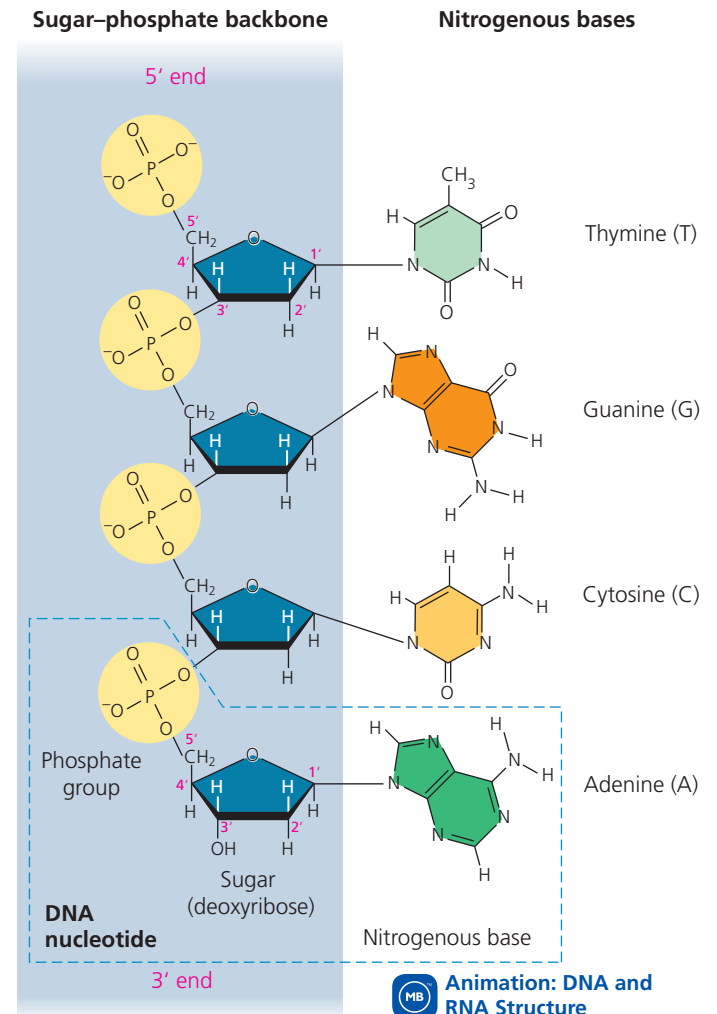
These two findings became known as *Chargaff's rules*:

- (1) DNA base composition varies between species, and
- (2) for each species, the percentages of A and T bases are roughly equal, as are those of G and C bases. In the **Scientific Skills Exercise**, you can use Chargaff's rules to predict percentages of nucleotide bases. The basis for these rules remained unexplained until the discovery of the double helix.

Building a Structural Model of DNA: Scientific Inquiry

Once most biologists were convinced that DNA was the genetic material, the challenge was to determine how the structure of DNA could account for its role in inheritance.

Figure 16.5 The structure of a DNA strand. Each DNA nucleotide monomer consists of a nitrogenous base (T, A, C, or G), the sugar deoxyribose (blue), and a phosphate group (yellow). The phosphate group of one nucleotide is attached to the sugar of the next by a covalent bond, forming a “backbone” of alternating phosphates and sugars from which the bases project. A polynucleotide strand has directionality, from the 5' end (with the phosphate group) to the 3' end (with the —OH group of the sugar). 5' and 3' refer to the numbers assigned to the carbons in the sugar ring.



By the early 1950s, the arrangement of covalent bonds in a nucleic acid polymer was well established (see Figure 16.5), and researchers focused on discovering the three-dimensional structure of DNA. Among the scientists working on the problem were Linus Pauling, at the California Institute of Technology, and Maurice Wilkins and Rosalind Franklin, at King's College in London. First to come up with the complete answer, however, were two scientists who were relatively unknown at the time—the American James Watson and the Englishman Francis Crick.

The brief but celebrated partnership that solved the puzzle of DNA structure began soon after Watson journeyed to Cambridge University, where Crick was studying protein structure with a technique called X-ray crystallography (see Figure 5.21). While visiting the laboratory of

SCIENTIFIC SKILLS EXERCISE

Working with Data in a Table

Given the Percentage Composition of One Nucleotide in a Genome, Can We Predict the Percentages of the Other Three Nucleotides? Even before the structure of DNA was elucidated, Erwin Chargaff and his co-workers noticed a pattern in the base composition of nucleotides from different species: The percentage of adenine (A) bases roughly equaled that of thymine (T) bases, and the percentage of cytosine (C) bases roughly equaled that of guanine (G) bases. Further, the percentage of each pair (A/T or C/G) varied from species to species. We now know that the 1:1 A/T and C/G ratios are due to complementary base pairing between A and T and between C and G in the DNA double helix, and interspecies differences are due to the unique sequences of bases along a DNA strand. In this exercise, you will apply Chargaff's rules to predict the composition of bases in a genome.

How the Experiments Were Done In Chargaff's experiments, DNA was extracted from the given species, hydrolyzed to break apart the nucleotides, and then analyzed chemically. These studies provided approximate values for each type of nucleotide. (Today, whole-genome sequencing allows base composition analysis to be done more precisely directly from the sequence data.)

Data from the Experiments Tables are useful for organizing sets of data representing a common set of values (here, percentages of A, G, C, and T) for a number of different samples (in this case, from different species). You can apply the patterns that you see in the known data to predict unknown values. In the table, complete base distribution data are given for sea urchin DNA and salmon DNA; you will use Chargaff's rules to fill in the rest of the table with predicted values.

Source of DNA	Base Percentage			
	Adenine	Guanine	Cytosine	Thymine
Sea urchin	32.8	17.7	17.3	32.1
Salmon	29.7	20.8	20.4	29.1
Wheat	28.1	21.8	22.7	
<i>E. coli</i>	24.7	26.0		
Human	30.4			30.1
Ox	29.0			
Average %				

Data from several papers by Chargaff; for example, E. Chargaff et al., Composition of the desoxyribose nucleic acids of four genera of sea-urchin, *Journal of Biological Chemistry* 195:155–160 (1952).

➤ **Sea urchin**



INTERPRET THE DATA

1. Explain how the sea urchin and salmon data demonstrate both of Chargaff's rules.
2. Using Chargaff's rules, fill in the table with your predictions of the missing percentages of bases, starting with the wheat genome and proceeding through *E. coli*, human, and ox. Show how you arrived at your answers.
3. If Chargaff's rule—that the amount of A equals the amount of T and the amount of C equals the amount of G—is valid, then hypothetically we could extrapolate this to the combined DNA of all species on Earth (like one huge Earth genome). To see whether the data in the table support this hypothesis, calculate the average percentage for each base in your completed table by averaging the values in each column. Does Chargaff's equivalence rule still hold true?

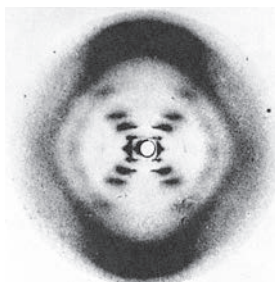


Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Maurice Wilkins, Watson saw an X-ray diffraction image of DNA produced by Wilkins's accomplished colleague Rosalind Franklin (**Figure 16.6**). Images produced by X-ray crystallography are not actually pictures of molecules. The spots and smudges in the image were produced by X-rays that were diffracted (deflected) as they passed through aligned fibers of purified DNA. Watson was familiar with the type of X-ray diffraction pattern that helical molecules produce, and an examination of the photo that Wilkins showed him confirmed that DNA was helical in shape. The photo also augmented earlier data obtained by Franklin and others suggesting the width of the helix and the spacing of



◀ **Figure 16.6** Rosalind Franklin and her X-ray diffraction photo of DNA.



the nitrogenous bases along it. The pattern in this photo implied that the helix was made up of two strands, contrary to a three-stranded model that Linus Pauling had proposed a short time earlier. The presence of two strands accounts for the now-familiar term **double helix**. DNA is shown in some of its many different representations in **Figure 16.7**.

Watson and Crick began building models of a double helix that would conform to the X-ray measurements and what was then known about the chemistry of DNA, including Chargaff's rule of base equivalences. Having also read an unpublished annual report summarizing Franklin's work, they knew she had concluded that the sugar-phosphate backbones were on the outside of the DNA molecule, contrary to their working model. Franklin's arrangement was appealing because it put the negatively charged phosphate groups facing the aqueous surroundings, while the relatively hydrophobic nitrogenous bases were hidden in the interior. Watson constructed such a model, shown in the small photo on the first page of this chapter. In this model, the two sugar-phosphate backbones are **antiparallel**—that is, their subunits run in opposite directions (see Figure 16.7). You can imagine the overall arrangement as a rope ladder with rigid rungs. The side ropes represent the sugar-phosphate backbones, and the rungs represent pairs of nitrogenous bases. Now imagine twisting the

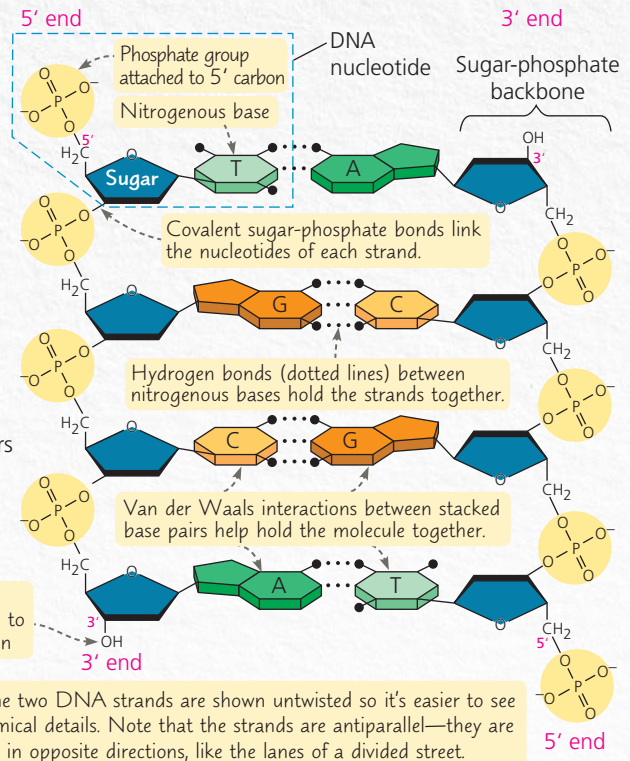
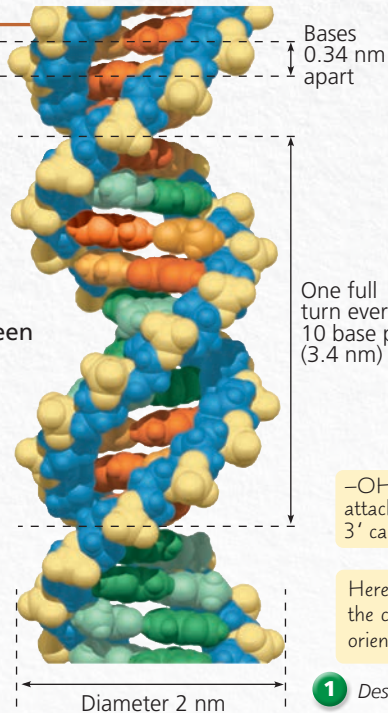
▼ Figure 16.7 Visualizing DNA

DNA can be illustrated in many ways, but all diagrams represent the same basic structure. The level of detail shown depends on the process or the type of information being conveyed.

Structural Images

These structural images show the three-dimensional shape of the DNA double helix (left) and chemical details of DNA's structure (right). Both images use the same colors for phosphate groups (yellow), deoxyribose sugars (blue), and nitrogenous bases (shades of green and orange).

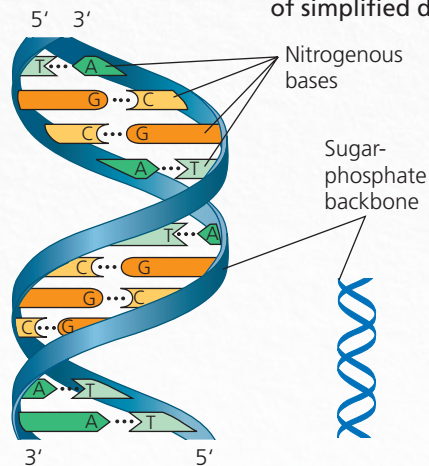
The DNA double helix is right-handed, as shown in this computer-generated space-filling model. Use your right hand as shown to follow the sugar-phosphate backbone up the helix (red arrow) and around to the back. (It won't work with your left hand.)



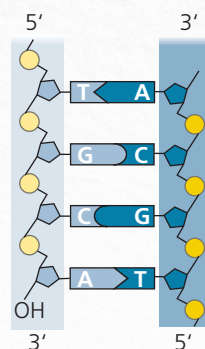
1 Describe the bonds that hold together the nucleotides in one DNA strand. Then compare them with the bonds that hold the two DNA strands together.

Simplified Images

When molecular detail is not necessary, DNA is portrayed in a range of simplified diagrams, depending on the focus of the figure.

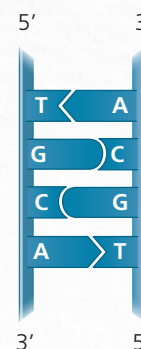


The "ribbons" in these simplified double helix diagrams represent the sugar-phosphate backbones.



These flattened "ladder style" diagrams of DNA depict the sugar-phosphate backbones like the side rails of a ladder, with the base pairs as rungs. Light blue is used to indicate the more recently synthesized strand.

2 Compare the information conveyed in the three ladder diagrams.



Sometimes the double-stranded DNA molecule is shown simply as two straight lines.

DNA Sequences

Genetic information is carried in DNA as a linear sequence of nucleotides that may be transcribed into mRNA and translated into a polypeptide. When focusing on the DNA sequence, each nucleotide can be represented simply as the letter of its base: A, T, C, or G.

3' - A C G T A A G C G G T T A A T - 5'
5' - T G C A T T C G C C A A T T A - 3'



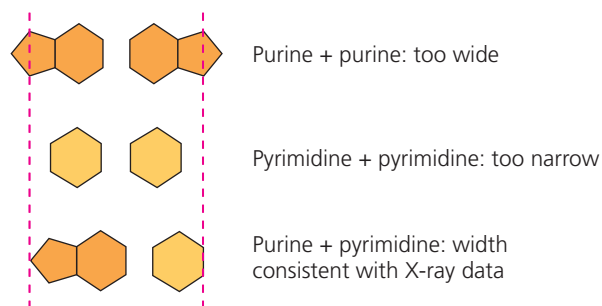
Instructors: Additional questions related to this Visualizing Figure can be assigned in MasteringBiology.



Animation: DNA Double Helix

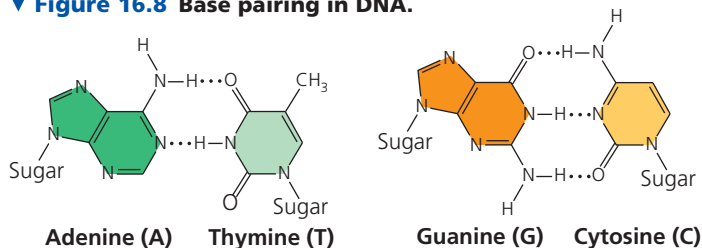
ladder to form a helix. Franklin's X-ray data indicated that the helix makes one full turn every 3.4 nm along its length. With the bases stacked just 0.34 nm apart, there are ten layers of base pairs, or rungs of the ladder, in each full turn of the helix.

The nitrogenous bases of the double helix are paired in specific combinations: adenine (A) with thymine (T), and guanine (G) with cytosine (C). It was mainly by trial and error that Watson and Crick arrived at this key feature of DNA. At first, Watson imagined that the bases paired like with like—for example, A with A and C with C. But this model did not fit the X-ray data, which suggested that the double helix had a uniform diameter. Why is this requirement inconsistent with like-with-like pairing of bases? Adenine and guanine are purines, nitrogenous bases with two organic rings, while cytosine and thymine are nitrogenous bases called pyrimidines, which have a single ring. Pairing a purine with a pyrimidine is the only combination that results in a uniform diameter for the double helix:



Watson and Crick reasoned that there must be additional specificity of pairing dictated by the structure of the bases. Each base has chemical side groups that can form hydrogen bonds with its appropriate partner: Adenine can form two hydrogen bonds with thymine and only thymine; guanine forms three hydrogen bonds with cytosine and only cytosine. In shorthand, A pairs with T, and G pairs with C (**Figure 16.8**).

▼ **Figure 16.8** Base pairing in DNA.



The Watson-Crick model took into account Chargaff's ratios and ultimately explained them. Wherever one strand of a DNA molecule has an A, the partner strand has a T. Similarly, a G in one strand is always paired with a C in the complementary strand. Therefore, in the DNA of any organism, the amount of adenine equals the amount of thymine, and the amount of guanine equals the amount of cytosine. (Modern DNA sequencing techniques have confirmed that the amounts are exactly equal.) Although the base-pairing rules dictate the combinations of nitrogenous bases that

form the “rungs” of the double helix, they do not restrict the sequence of nucleotides *along* each DNA strand. The linear sequence of the four bases can be varied in countless ways, and each gene has a unique base sequence.

In April 1953, Watson and Crick surprised the scientific world with a succinct, one-page paper that reported their molecular model for DNA: the double helix, which has since become the symbol of molecular biology. Watson and Crick, along with Maurice Wilkins, were awarded the Nobel Prize in 1962 for this work. (Sadly, Rosalind Franklin had died at the age of 37 in 1958 and was thus ineligible for the prize.) The beauty of the double helix model was that the structure of DNA suggested the basic mechanism of its replication.

 **HHMI Video: Great Discoveries in Science: The Double Helix**

 **hhmi**
BioInteractive

CONCEPT CHECK 16.1

1. Given a polynucleotide sequence such as GAATTC, explain what further information you would need in order to identify which is the 5' end. (See Figure 16.5.)
2. **VISUAL SKILLS** ► Griffith was trying to develop a vaccine for *S. pneumonia* when he was surprised to discover the phenomenon of bacterial transformation. Look at the second and third panels of Figure 16.2. Based on these results, what result was he expecting in the fourth panel? Explain.

For suggested answers, see Appendix A.

CONCEPT 16.2

Many proteins work together in DNA replication and repair

The relationship between structure and function is manifest in the double helix. The idea that there is specific pairing of nitrogenous bases in DNA was the flash of inspiration that led Watson and Crick to the double helix. At the same time, they saw the functional significance of the base-pairing rules. They ended their classic paper with this wry statement: “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”* In this section, you will learn about the basic principle of DNA replication, as well as some important details of the process.

The Basic Principle: Base Pairing to a Template Strand

In a second paper, Watson and Crick stated their hypothesis for how DNA replicates: “Now our model for deoxyribonucleic acid is, in effect, a pair of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for

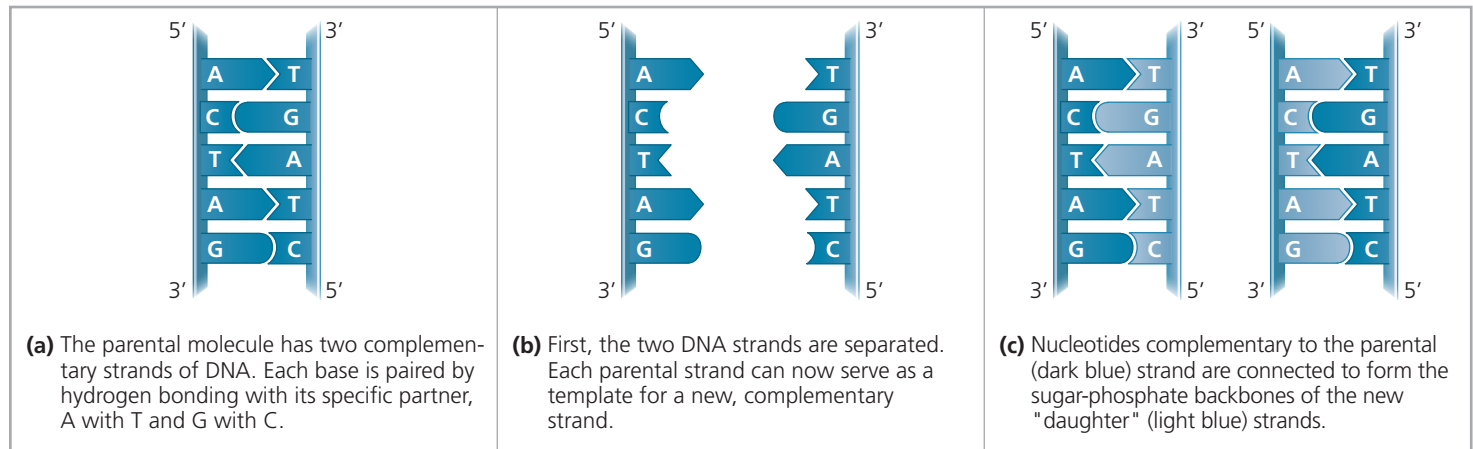
*J. D. Watson and F. H. C. Crick, Molecular structure of nucleic acids: a structure for deoxyribose nucleic acids, *Nature* 171:737–738 (1953).

▼ **Figure 16.9 A model for DNA replication: the basic concept.** In this simplified illustration, a short segment of DNA

has been untwisted. Simple shapes symbolize the four kinds of bases. Dark blue represents DNA strands present in the parental molecule;

light blue represents newly synthesized DNA.

 **Animation: DNA Replication: An Overview**



the formation on to itself of a new companion chain, so that eventually we shall have two pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly.”[†]

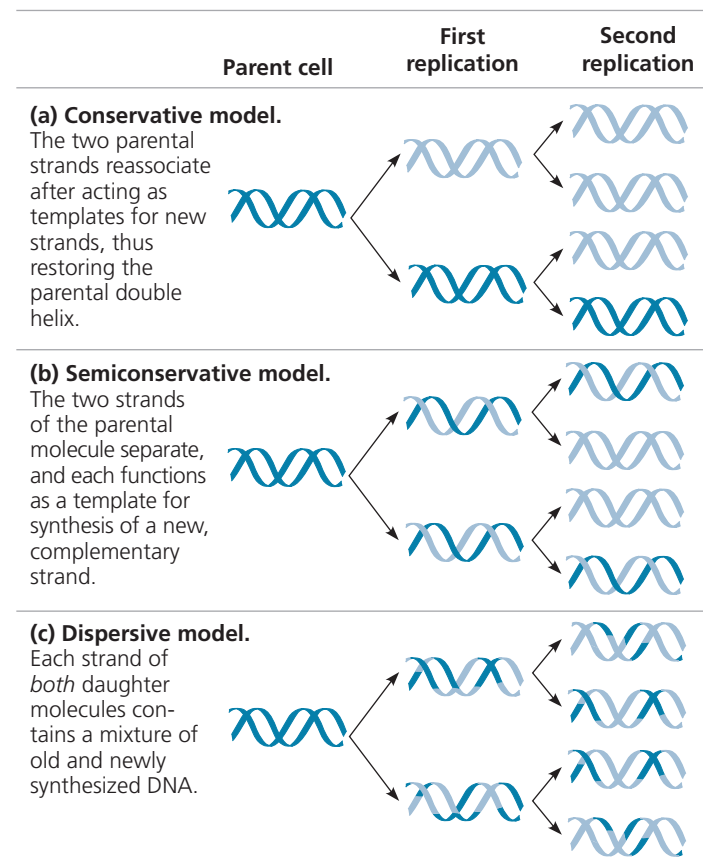
Figure 16.9 illustrates Watson and Crick’s basic idea.

To make it easier to follow, we show only a short section of double helix in untwisted form. Notice that if you cover one of the two DNA strands of Figure 16.9a, you can still determine its linear sequence of nucleotides by referring to the uncovered strand and applying the base-pairing rules. The two strands are complementary; each stores the information necessary to reconstruct the other. When a cell copies a DNA molecule, each strand serves as a template for ordering nucleotides into a new, complementary strand. Nucleotides line up along the template strand according to the base-pairing rules and are linked to form the new strands. Where there was one double-stranded DNA molecule at the beginning of the process, there are soon two, each an exact replica of the “parental” molecule. The copying mechanism is analogous to using a photographic negative to make a positive image, which can in turn be used to make another negative, and so on.

This model of DNA replication remained untested for several years following publication of the DNA structure. The requisite experiments were simple in concept but difficult to perform. Watson and Crick’s model predicts that when a double helix replicates, each of the two daughter molecules will have one old strand, from the parental molecule, and one newly made strand. This **semiconservative model** can be distinguished from a conservative model of replication, in which the two parental strands somehow come back together after the process (that is, the parental molecule is conserved). In yet a third model, called the dispersive model, all four strands of DNA following replication have a mixture of old and new DNA (**Figure 16.10**).

▼ **Figure 16.10 DNA replication: three alternative models.**

Each short segment of double helix symbolizes the DNA within a cell. Beginning with a parent cell, we follow the DNA for two more generations of cells—two rounds of DNA replication. Parental DNA is dark blue; newly made DNA is light blue.



Although mechanisms for conservative or dispersive DNA replication are not easy to devise, these models remained possibilities until they could be ruled out. After two years of preliminary work at the California Institute of Technology in the late 1950s, Matthew Meselson and Franklin Stahl devised

[†]J. D. Watson and F. H. C. Crick, Genetical implications of the structure of deoxyribonucleic acid, *Nature* 171:964–967 (1953).

a clever experiment that distinguished between the three models, described in **Figure 16.11**. Their results supported the semiconservative model of DNA replication, as predicted by Watson and Crick, and their experiment is widely recognized among biologists as a classic example of elegant design.

The basic principle of DNA replication is conceptually simple. However, the actual process involves some complicated biochemical gymnastics, as we will now see.

DNA Replication: A Closer Look

The bacterium *E. coli* has a single chromosome of about 4.6 million nucleotide pairs. In a favorable environment, an *E. coli* cell can copy all of this DNA and divide to form two genetically identical daughter cells in considerably less than an hour. Each of *your* somatic cells has 46 DNA molecules in its nucleus, one long double-helical molecule per chromosome. In all, that represents about 6 billion nucleotide pairs, or over 1,000 times more DNA than is found in most bacterial cells. If we were to print the one-letter symbols for these bases (A, G, C, and T) the size of the type you are now reading, the 6 billion nucleotide pairs of information in a diploid human cell would fill about 1,400 biology textbooks. Yet it takes one of your cells just a few hours to copy all of this DNA during S phase of interphase. This replication of an enormous amount of genetic information is achieved with very few errors—only about one per 10 billion nucleotides. The copying of DNA is remarkable in its speed and accuracy.

More than a dozen enzymes and other proteins participate in DNA replication. Much more is known about how this “replication machine” works in bacteria (such as *E. coli*) than in eukaryotes, and we will describe the basic steps of the process for *E. coli*, except where otherwise noted. What scientists have learned about eukaryotic DNA replication suggests, however, that most of the process is fundamentally similar for prokaryotes and eukaryotes.

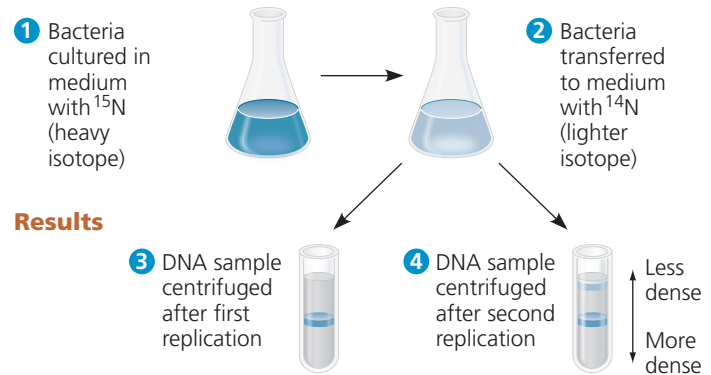
Getting Started

The replication of chromosomal DNA begins at particular sites called **origins of replication**, short stretches of DNA that have a specific sequence of nucleotides. The *E. coli* chromosome, like many other bacterial chromosomes, is circular and has a single origin. Proteins that initiate DNA replication recognize this sequence and attach to the DNA, separating the two strands and opening up a replication “bubble” (**Figure 16.12a**). Replication of DNA then proceeds in both directions until the entire molecule is copied. In contrast to a bacterial chromosome, a eukaryotic chromosome may have hundreds or even a few thousand replication origins. Multiple replication bubbles form and eventually fuse, thus speeding up the copying of the very long DNA molecules (**Figure 16.12b**). As in bacteria, eukaryotic DNA replication proceeds in both directions from each origin.

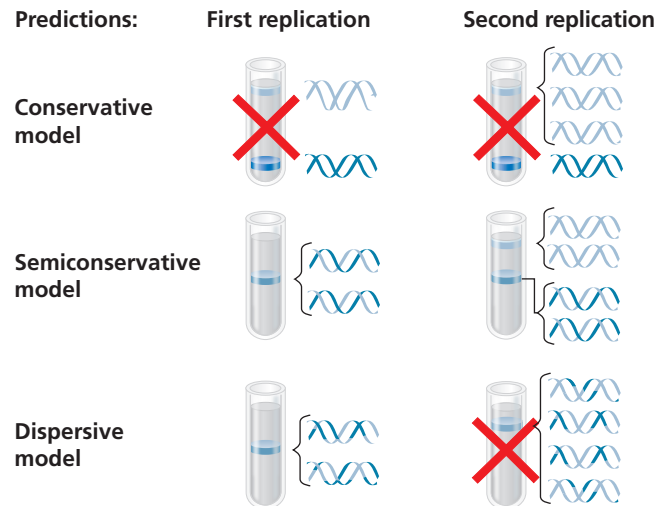
▼ Figure 16.11

Inquiry Does DNA replication follow the conservative, semiconservative, or dispersive model?

Experiment Matthew Meselson and Franklin Stahl cultured *E. coli* for several generations in a medium containing nucleotide precursors labeled with a heavy isotope of nitrogen, ^{15}N . They then transferred the bacteria to a medium with only ^{14}N , a lighter isotope. They took one sample after the first DNA replication and another after the second replication. They extracted DNA from the bacteria in the samples and then centrifuged each DNA sample to separate DNA of different densities.



Conclusion Meselson and Stahl compared their results to those predicted by each of the three models in Figure 16.10, as shown below. The first replication in the ^{14}N medium produced a band of hybrid (^{15}N - ^{14}N) DNA. This result eliminated the conservative model. The second replication produced both light and hybrid DNA, a result that refuted the dispersive model and supported the semiconservative model. They therefore concluded that DNA replication is semiconservative.



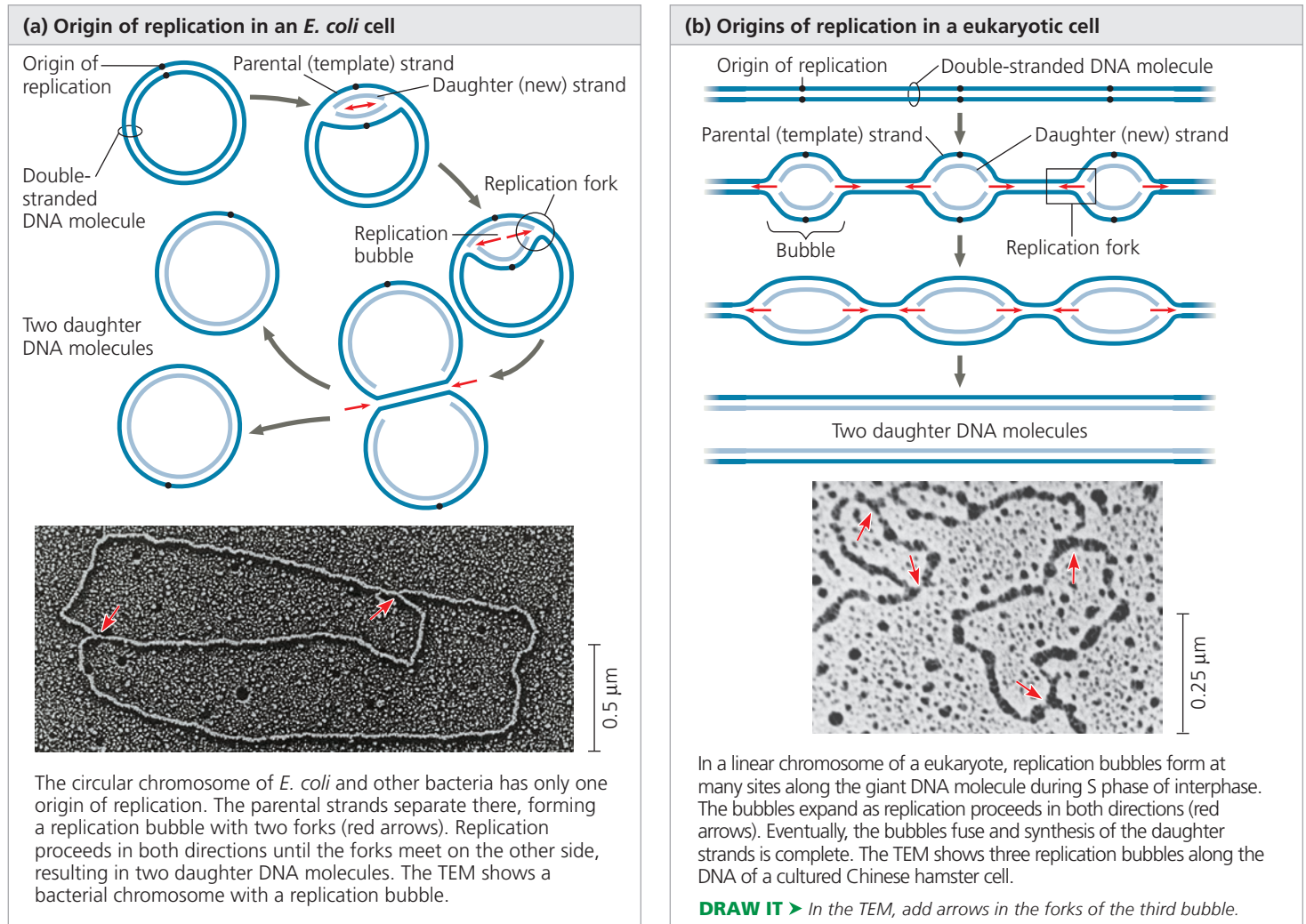
Data from M. Meselson and F. W. Stahl, The replication of DNA in *Escherichia coli*, *Proceedings of the National Academy of Sciences USA* 44:671–682 (1958).

Inquiry in Action Read and analyze the original paper in *Inquiry in Action: Interpreting Scientific Papers*.

Instructors: A related Experimental Inquiry Tutorial can be assigned in MasteringBiology.

WHAT IF? > If Meselson and Stahl had first grown the cells in ^{14}N -containing medium and then moved them into ^{15}N -containing medium before taking samples, what would have been the result after each of the two replications?

▼ **Figure 16.12 Origins of replication in *E. coli* and eukaryotes.** The red arrows indicate the movement of the replication forks and thus the overall directions of DNA replication within each bubble.



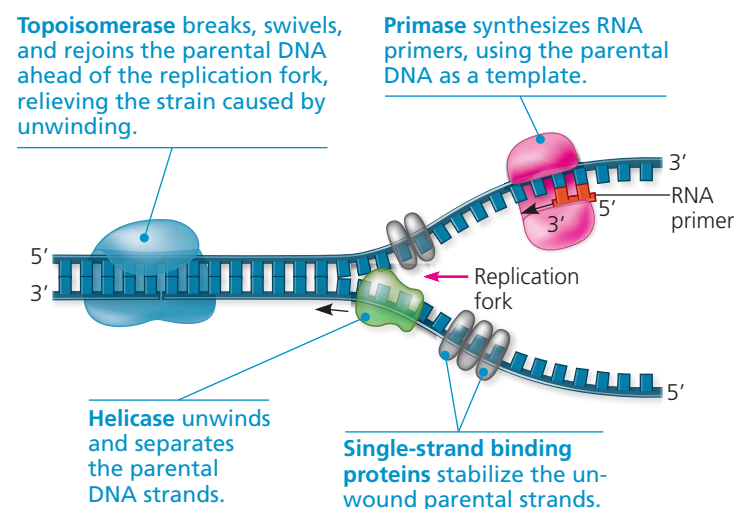
BioFlix® Animation: The Replication Fork in *E. coli*

At each end of a replication bubble is a **replication fork**, a Y-shaped region where the parental strands of DNA are being unwound. Several kinds of proteins participate in the unwinding (**Figure 16.13**). **Helicases** are enzymes that untwist the double helix at the replication forks, separating the two parental strands and making them available as template strands. After the parental strands separate, **single-strand binding proteins** bind to the unpaired DNA strands, keeping them from re-pairing. The untwisting of the double helix causes tighter twisting and strain ahead of the replication fork. **Topoisomerase** is an enzyme that helps relieve this strain by breaking, swiveling, and rejoining DNA strands.

Synthesizing a New DNA Strand

The unwound sections of parental DNA strands are now available to serve as templates for the synthesis of new complementary DNA strands. However, the enzymes that synthesize

▼ **Figure 16.13 Some of the proteins involved in the initiation of DNA replication.** The same proteins function at both replication forks in a replication bubble. For simplicity, only the left-hand fork is shown, and the DNA bases are drawn much larger in relation to the proteins than they are in reality.



DNA cannot *initiate* the synthesis of a polynucleotide; they can only add DNA nucleotides to the end of an already existing chain that is base-paired with the template strand. The initial nucleotide chain that is produced during DNA synthesis is actually a short stretch of RNA, not DNA. This RNA chain is called a **primer** and is synthesized by the enzyme **primase** (see Figure 16.13). Primase starts a complementary RNA chain with a single RNA nucleotide and adds RNA nucleotides one at a time, using the parental DNA strand as a template. The completed primer, generally 5–10 nucleotides long, is thus base-paired to the template strand. The new DNA strand will start from the 3' end of the RNA primer.

Enzymes called **DNA polymerases** catalyze the synthesis of new DNA by adding nucleotides to the 3' end of a pre-existing chain. In *E. coli*, there are several DNA polymerases, but two appear to play the major roles in DNA replication: DNA polymerase III and DNA polymerase I. The situation in eukaryotes is more complicated, with at least 11 different DNA polymerases discovered so far, although the general principles are the same.

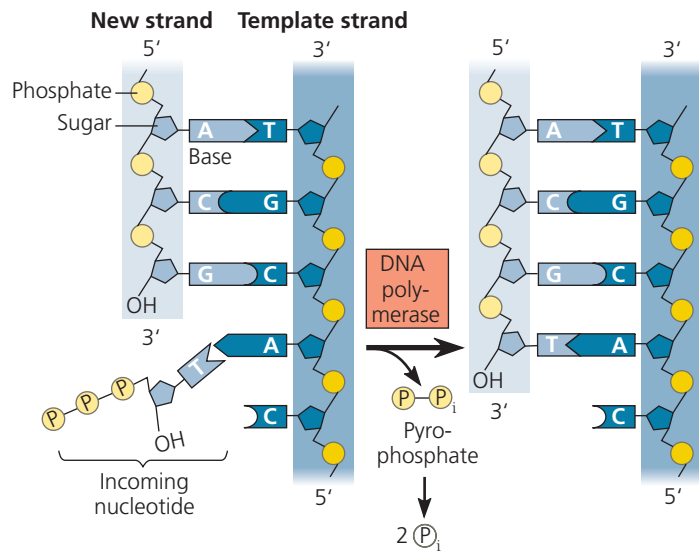
Most DNA polymerases require a primer and a DNA template strand, along which complementary DNA nucleotides are lined up. In *E. coli*, DNA polymerase III (abbreviated DNA pol III) adds a DNA nucleotide to the RNA primer and then continues adding DNA nucleotides, complementary to the parental DNA template strand, to the growing end of the new DNA strand. The rate of elongation is about 500 nucleotides per second in bacteria and 50 per second in human cells.

Each nucleotide to be added to a growing DNA strand consists of a sugar attached to a base and to three phosphate groups. You have already encountered such a molecule—ATP (adenosine triphosphate; see Figure 6.9). The only difference between the ATP of energy metabolism and dATP, the adenine nucleotide used to make DNA, is the sugar component, which is deoxyribose in the building block of DNA but ribose in ATP. Like ATP, the nucleotides used for DNA synthesis are chemically reactive, partly because their triphosphate tails have an unstable cluster of negative charge. DNA polymerase catalyzes the addition of each monomer via a dehydration reaction (see Figure 5.2a). As each monomer is joined to the growing end of a DNA strand, two phosphate groups are lost as a molecule of pyrophosphate ($\text{P}-\text{P}_i$). Subsequent hydrolysis of the pyrophosphate to two molecules of inorganic phosphate (P_i) is a coupled exergonic reaction that helps drive the polymerization reaction (Figure 16.14).

Antiparallel Elongation

As we have noted previously, the two ends of a DNA strand are different, giving each strand directionality, like a one-way street (see Figure 16.5). In addition, the two strands of DNA in a double helix are antiparallel, meaning that they are oriented in opposite directions to each other, like the two sides of a divided street (see Figure 16.14). Therefore, the two

▼ **Figure 16.14 Addition of a nucleotide to a DNA strand.** DNA polymerase catalyzes the addition of a nucleotide to the 3' end of a growing DNA strand, with the release of two phosphates.



VISUAL SKILLS ► Use this diagram to explain what we mean when we say that each DNA strand has directionality.

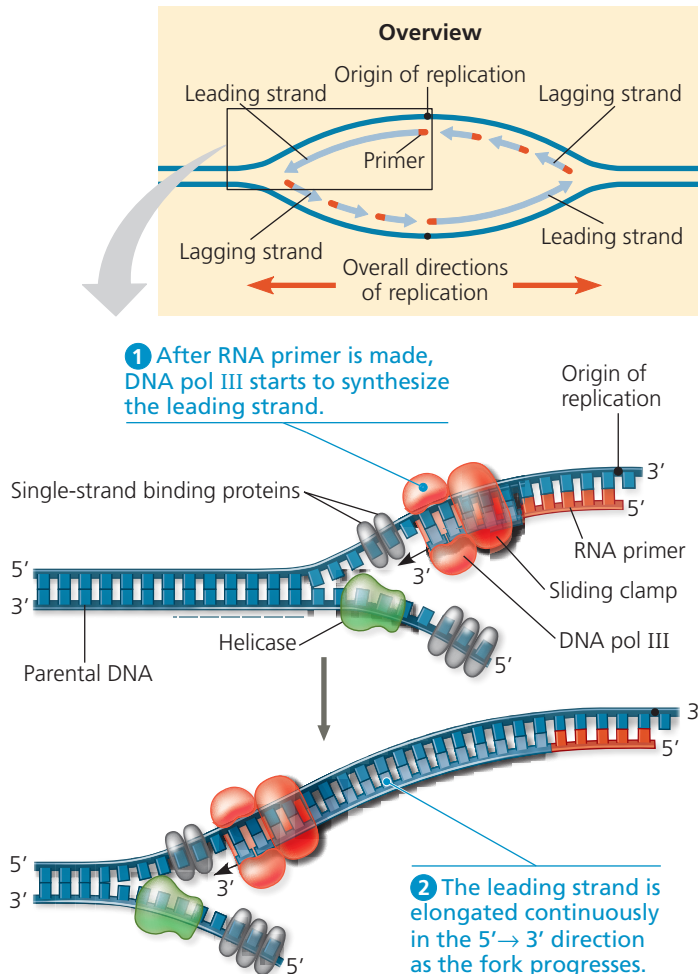
Figure Walkthrough

new strands formed during DNA replication must also be antiparallel to their template strands.

The antiparallel arrangement of the double helix, together with a property of DNA polymerases, has an important effect on how replication occurs. Because of their structure, DNA polymerases can add nucleotides only to the free 3' end of a primer or growing DNA strand, never to the 5' end (see Figure 16.14). Thus, a new DNA strand can elongate only in the 5' → 3' direction. With this in mind, let's examine one of the two replication forks in a bubble (Figure 16.15). Along one template strand, DNA polymerase III can synthesize a complementary strand continuously by elongating the new DNA in the mandatory 5' → 3' direction. DNA pol III remains in the replication fork on that template strand and continuously adds nucleotides to the new complementary strand as the fork progresses. The DNA strand made by this mechanism is called the **leading strand**. Only one primer is required for DNA pol III to synthesize the entire leading strand (see Figure 16.15).

To elongate the other new strand of DNA in the mandatory 5' → 3' direction, DNA pol III must work along the other template strand in the direction *away from* the replication fork. The DNA strand elongating in this direction is called the **lagging strand**. In contrast to the leading strand, which elongates continuously, the lagging strand is synthesized discontinuously, as a series of segments. These segments of the lagging strand are called **Okazaki fragments**, after Reiji Okazaki, the Japanese scientist who discovered them. The fragments are about 1,000–2,000 nucleotides long in *E. coli* and 100–200 nucleotides long in eukaryotes.

▼ **Figure 16.15 Synthesis of the leading strand during DNA replication.** This diagram focuses on the left replication fork shown in the overview box. DNA polymerase III (DNA pol III), shaped like a cupped hand, is shown closely associated with a protein called the “sliding clamp” that encircles the newly synthesized double helix like a doughnut. The sliding clamp moves DNA pol III along the DNA template strand.

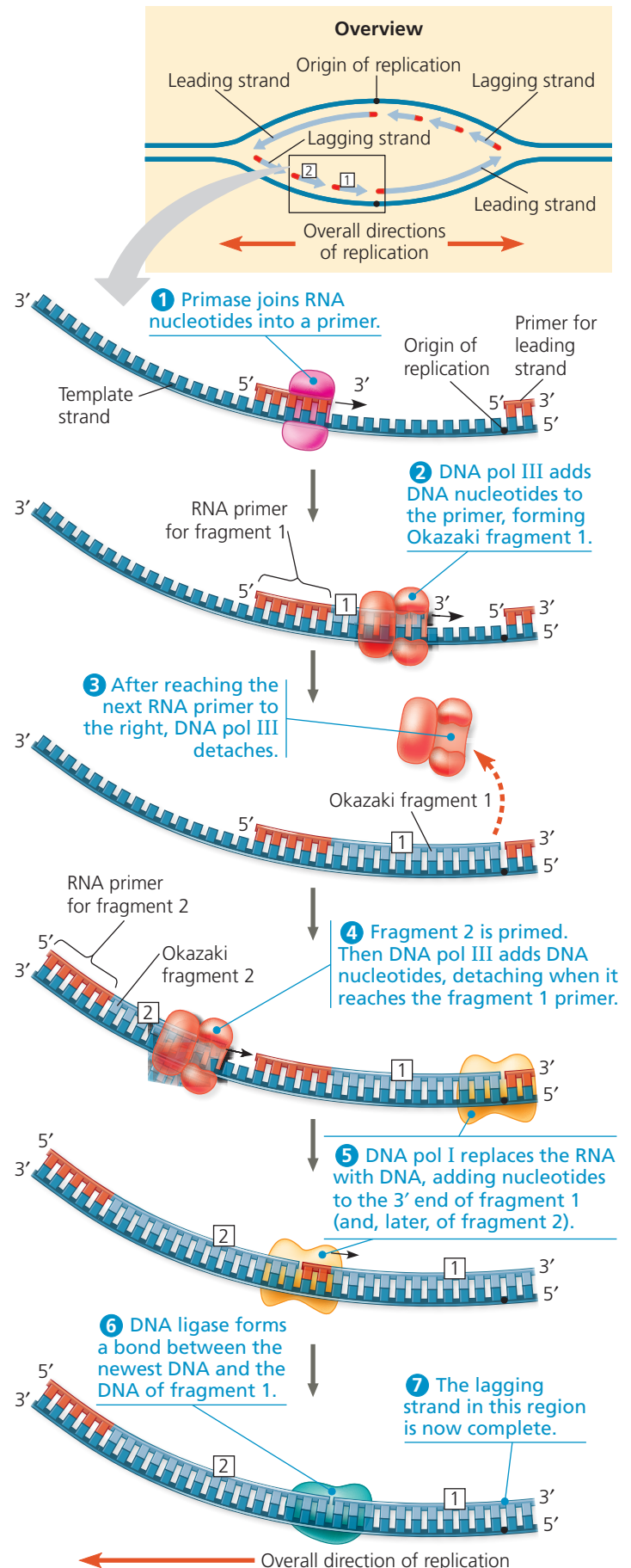


BioFlix® Animation: Synthesis of the Leading Strand

Figure 16.16 illustrates the steps in the synthesis of the lagging strand at one fork. Whereas only one primer is required on the leading strand, each Okazaki fragment on the lagging strand must be primed separately (steps 1 and 4). After DNA pol III forms an Okazaki fragment (steps 2 to 4), another DNA polymerase, DNA pol I, replaces the RNA nucleotides of the adjacent primer with DNA nucleotides one at a time (step 5). But DNA pol I cannot join the final nucleotide of this replacement DNA segment to the first DNA nucleotide of the adjacent Okazaki fragment. Another enzyme, **DNA ligase**, accomplishes this task, joining the sugar-phosphate backbones of all the Okazaki fragments into a continuous DNA strand (step 6).

BioFlix® Animation: Synthesis of the Lagging Strand

▼ **Figure 16.16 Synthesis of the lagging strand.**



Synthesis of the leading strand and synthesis of the lagging strand occur concurrently and at the same rate. The lagging strand is so named because its synthesis is delayed slightly relative to synthesis of the leading strand; each new fragment of the lagging strand cannot be started until enough template has been exposed at the replication fork.

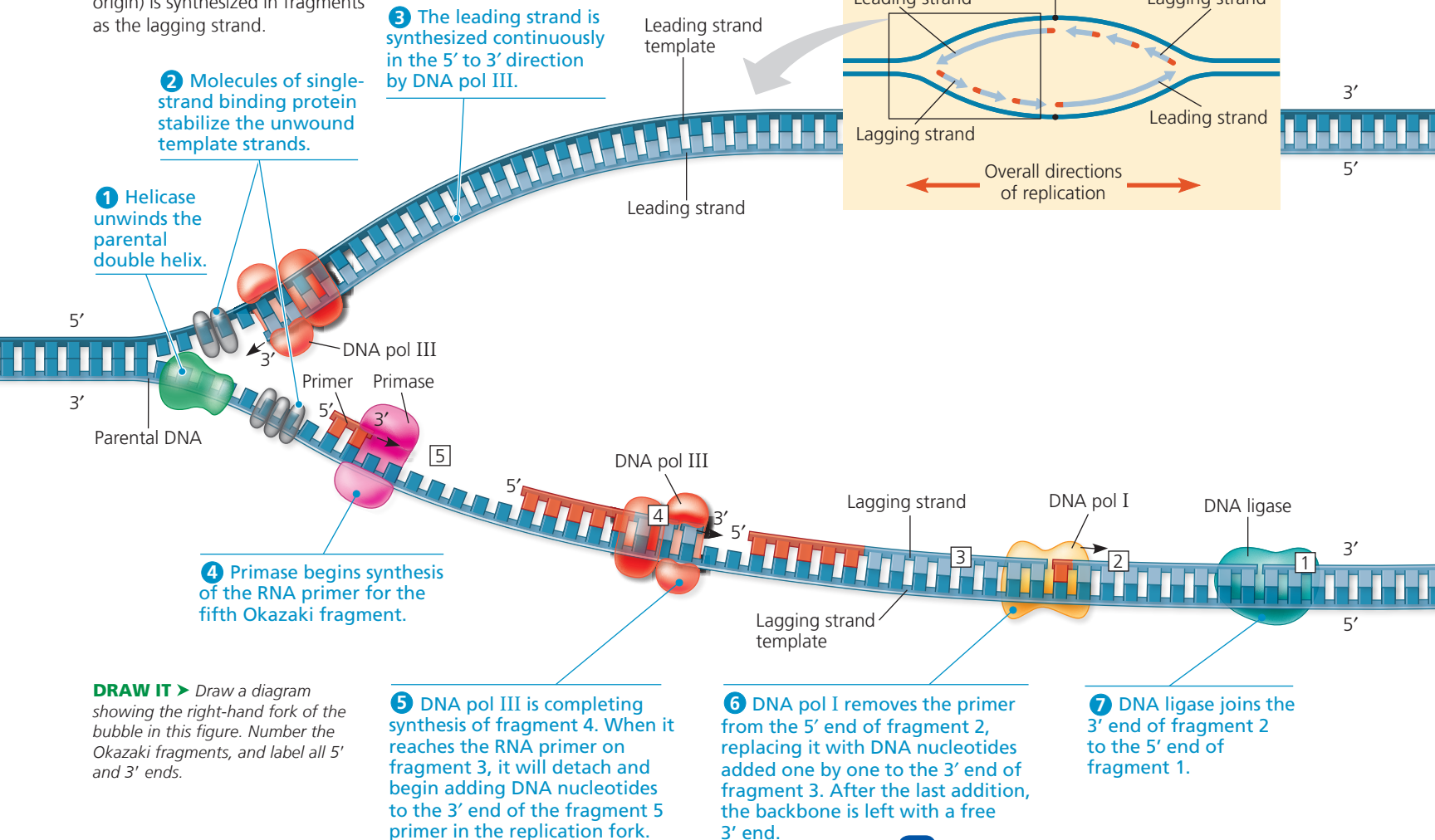
Figure 16.17 and **Table 16.1** summarize DNA replication. Please study them carefully before proceeding.

The DNA Replication Complex

It is traditional—and convenient—to represent DNA polymerase molecules as locomotives moving along a DNA railroad track, but such a model is inaccurate in two important ways. First, the various proteins that participate in DNA replication actually form a single large complex, a “DNA replication machine.” Many protein-protein interactions facilitate the efficiency of this complex. For example, by interacting with other proteins at the fork, primase apparently acts as a

molecular brake, slowing progress of the replication fork and coordinating the placement of primers and the rates of replication on the leading and lagging strands. Second, the DNA replication complex may not move along the DNA; rather, the DNA may move through the complex during the replication process. In eukaryotic cells, multiple copies of the complex, perhaps grouped into “factories,” may be anchored to the nuclear matrix, a framework of fibers extending through the interior of the nucleus. Experimental evidence in some type of cells supports a model in which two DNA polymerase molecules, one on each template strand, “reel in” the parental DNA and extrude newly made daughter DNA molecules. In this so-called trombone model, the lagging strand is also looped back through the complex (**Figure 16.18**). Whether the complex moves along the DNA or whether the DNA moves through the complex, either anchored or not, are still open, unresolved questions that are under active investigation. It is also possible that the process varies among species.

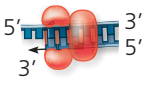
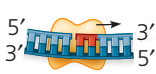
Figure 16.17 A summary of bacterial DNA replication. The detailed diagram shows the left-hand replication fork of the replication bubble shown in the overview (upper right). Viewing each daughter strand in its entirety in the overview, you can see that half of it is made continuously as the leading strand, while the other half (on the other side of the origin) is synthesized in fragments as the lagging strand.



DRAW IT ➤ Draw a diagram showing the right-hand fork of the bubble in this figure. Number the Okazaki fragments, and label all 5' and 3' ends.



Animation: DNA Replication: A Closer Look

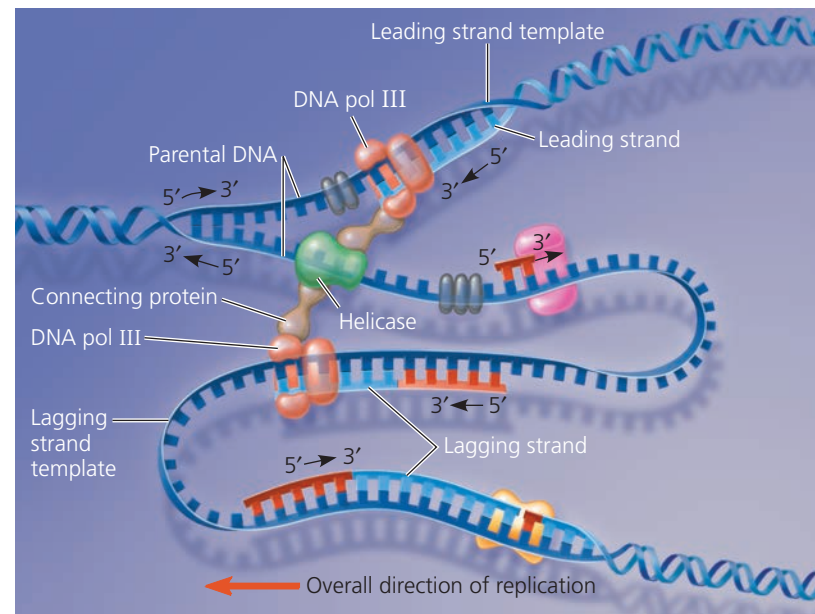
Table 16.1 Bacterial DNA Replication Proteins and Their Functions		
Protein		Function
Helicase		Unwinds parental double helix at replication forks
Single-strand binding protein		Binds to and stabilizes single-stranded DNA until it is used as a template
Topoisomerase		Relieves overwinding strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase		Synthesizes an RNA primer at 5' end of leading strand and at 5' end of each Okazaki fragment of lagging strand
DNA pol III		Using parental DNA as a template, synthesizes new DNA strand by adding nucleotides to an RNA primer or a pre-existing DNA strand
DNA pol I		Removes RNA nucleotides of primer from 5' end and replaces them with DNA nucleotides added to 3' end of adjacent fragment
DNA ligase		Joins Okazaki fragments of lagging strand; on leading strand, joins 3' end of DNA that replaces primer to rest of leading strand DNA

Proofreading and Repairing DNA

We cannot attribute the accuracy of DNA replication solely to the specificity of base pairing. Initial pairing errors between incoming nucleotides and those in the template strand occur at a rate of one in 10^5 nucleotides. However, errors in the completed DNA molecule amount to only one in 10^{10} (10 billion) nucleotides, an error rate that is 100,000 times lower. This is because during DNA replication, many DNA polymerases proofread each nucleotide against its template as soon as it is covalently bonded to the growing strand. Upon finding an incorrectly paired nucleotide, the polymerase removes the nucleotide and then resumes synthesis. (This action is similar to fixing a texting error by deleting the wrong letter and then entering the correct one.)

Mismatched nucleotides sometimes evade proofreading by a DNA polymerase. In **mismatch repair**, other enzymes remove and replace incorrectly paired nucleotides that have resulted from replication errors. Researchers highlighted the importance of such repair enzymes when they found that a hereditary defect in one of them is associated with a form of colon cancer. Apparently, this defect allows cancer-causing errors to accumulate in the DNA faster than normal.

▼ **Figure 16.18 The “trombone” model of the DNA replication complex.** In this proposed model, two molecules of DNA polymerase III work together in a complex, one on each strand, with helicase and other proteins. The lagging strand template DNA loops through the complex, resembling the slide of a trombone.



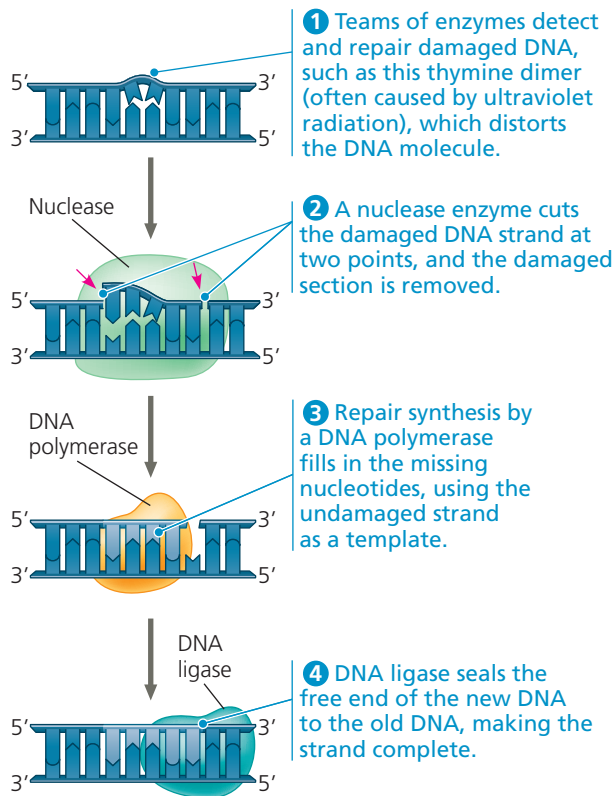
DRAW IT ➤ Draw a line tracing the lagging strand template along the entire stretch of DNA shown here.

 **BioFlix® Animation: The DNA Replication Complex**

Incorrectly paired or altered nucleotides can also arise after replication. In fact, maintenance of the genetic information encoded in DNA requires frequent repair of various kinds of damage to existing DNA. DNA molecules are constantly subjected to potentially harmful chemical and physical agents, such as X-rays, as we'll discuss in Concept 17.5. In addition, DNA bases may undergo spontaneous chemical changes under normal cellular conditions. However, these changes in DNA are usually corrected before they become permanent changes—**mutations**—perpetuated through successive replications. Each cell continuously monitors and repairs its genetic material. Because repair of damaged DNA is so important to the survival of an organism, it is no surprise that many different DNA repair enzymes have evolved. Almost 100 are known in *E. coli*, and about 170 have been identified so far in humans.

Most cellular systems for repairing incorrectly paired nucleotides, whether they are due to DNA damage or to replication errors, use a mechanism that takes advantage of the base-paired structure of DNA. In many cases, a segment of the strand containing the damage is cut out (excised) by a DNA-cutting enzyme—a **nuclease**—and the resulting gap is then filled in with nucleotides, using the undamaged strand as a template. The enzymes involved in filling the gap are a DNA polymerase and DNA ligase. One such DNA repair system is called **nucleotide excision repair** (Figure 16.19).

▼ **Figure 16.19** Nucleotide excision repair of DNA damage.



An important function of the DNA repair enzymes in our skin cells is to repair genetic damage caused by the ultraviolet rays of sunlight. One type of damage, shown in Figure 16.19, is the covalent linking of thymine bases that are adjacent on a DNA strand. Such *thymine dimers* cause the DNA to buckle and interfere with DNA replication. The importance of repairing this kind of damage is underscored by a disorder called xeroderma pigmentosum (XP), which in most cases is caused by an inherited defect in a nucleotide excision repair enzyme. Individuals with XP are hypersensitive to sunlight; mutations in their skin cells caused by ultraviolet light are left uncorrected, often resulting in skin cancer. The effects are extreme: Without sun protection, children who have XP can develop skin cancer by age 10.

Evolutionary Significance of Altered DNA Nucleotides

EVOLUTION Faithful replication of the genome and repair of DNA damage are important for the functioning of the organism and for passing on a complete, accurate genome to the next generation. The error rate after proofreading and repair is extremely low, but rare mistakes do slip through. Once a mismatched nucleotide pair is replicated, the sequence change is permanent in the daughter molecule that has the incorrect nucleotide as well as in any subsequent copies. As we mentioned earlier, a permanent change in the DNA sequence is called a mutation.

Mutations can change the phenotype of an organism (as you'll learn in Concept 17.5). And if they occur in germ cells, which give rise to gametes, mutations can be passed on from generation to generation. The vast majority of such changes either have no effect or are harmful, but a very small percentage can be beneficial. In either case, mutations are the original source of the variation on which natural selection operates during evolution and are ultimately responsible for the appearance of new species. (You'll learn more about this process in Unit Four.) The balance between complete fidelity of DNA replication or repair and a low mutation rate has resulted in new proteins that contribute to different phenotypes. Ultimately, over long periods of time, this process leads to new species and thus to the rich diversity of life we see on Earth today.

Replicating the Ends of DNA Molecules

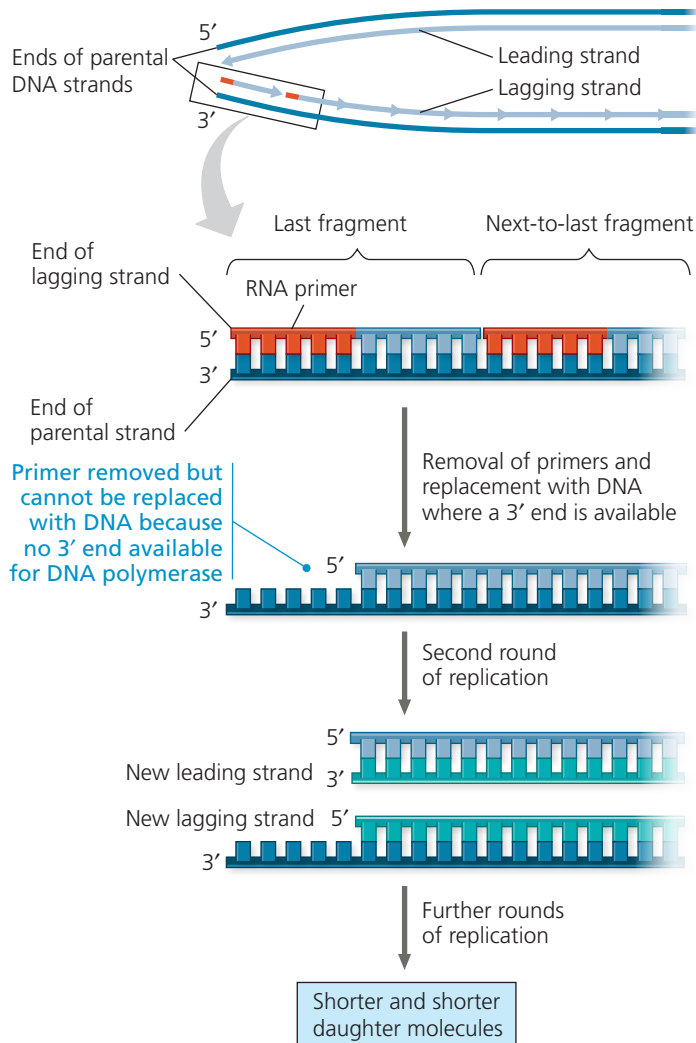
For linear DNA, such as the DNA of eukaryotic chromosomes, the usual replication machinery cannot complete the 5' ends of daughter DNA strands. (This is another consequence of the fact that a DNA polymerase can add nucleotides only to the 3' end of a preexisting polynucleotide.) Even if an Okazaki fragment can be started with an RNA primer hydrogen-bonded to the very end of the template strand, once that primer is removed, it cannot be replaced with DNA because there is no 3' end available for nucleotide addition (Figure 16.20). As a result, repeated rounds of replication produce shorter and shorter DNA molecules with uneven ("staggered") ends.

Most prokaryotes have a circular chromosome, with no ends, so the shortening of DNA does not occur. But what protects the genes of linear eukaryotic chromosomes from being eroded away during successive rounds of DNA replication? Eukaryotic chromosomal DNA molecules have special nucleotide sequences called **telomeres** at their ends (Figure 16.21). Telomeres do not contain genes; instead, the DNA typically consists of multiple repetitions of one short nucleotide sequence. In each human telomere, for example, the six-nucleotide sequence TTAGGG is repeated between 100 and 1,000 times.

Telomeres have two protective functions. First, specific proteins associated with telomeric DNA prevent the staggered ends of the daughter molecule from activating the cell's systems for monitoring DNA damage. (Staggered ends of a DNA molecule, which often result from double-strand breaks, can trigger signal transduction pathways leading to cell cycle arrest or cell death.) Second, telomeric DNA acts as a kind of buffer zone that provides some protection against the organism's genes shortening, somewhat like how the plastic-wrapped ends of a shoelace slow down its unraveling. Telomeres do not prevent the erosion of genes near the ends of chromosomes; they merely postpone it.

As shown in Figure 16.20, telomeres become shorter during every round of replication. Thus, as expected, telomeric DNA tends to be shorter in dividing somatic cells of older individuals and in cultured cells that have divided many

▼ **Figure 16.20 Shortening of the ends of linear DNA molecules.** Here we follow the left end of one DNA molecule through two rounds of replication. After the first round, the new lagging strand is shorter than its template. After a second round, both the leading and lagging strands have become shorter than the original parental DNA. Although not shown here, the other ends of these chromosomal DNA molecules (not shown) also become shorter.



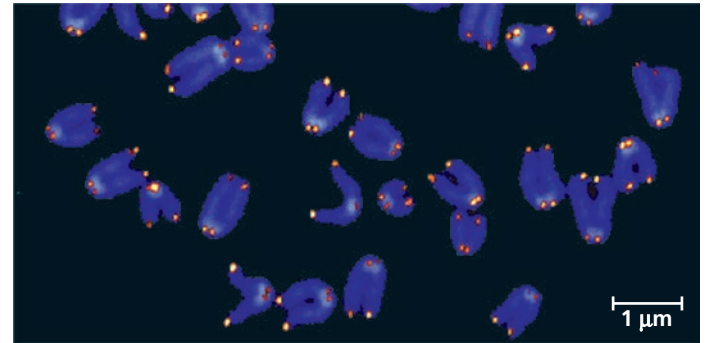
times. It has been proposed that shortening of telomeres is somehow connected to the aging process of certain tissues and even to aging of the organism as a whole.



ABC News Video: The Effect of Exercise on Cells

But what about cells whose genome must persist virtually unchanged from an organism to its offspring over many generations? If the chromosomes of germ cells became shorter in every cell cycle, essential genes would eventually be missing from the gametes they produce. However, this does not occur: An enzyme called *telomerase* catalyzes the lengthening of telomeres in eukaryotic germ cells, thus restoring their original length and compensating for the shortening that occurs during DNA replication. This enzyme contains its

▼ **Figure 16.21 Telomeres.** Eukaryotes have repetitive, noncoding sequences called telomeres at the ends of their DNA. Telomeres are stained orange in these mouse chromosomes (LM).



own RNA molecule that it uses as a template to artificially “extend” the leading strand, allowing the lagging strand to maintain a given length. Telomerase is not active in most human somatic cells, but its activity varies from tissue to tissue. The activity of telomerase in germ cells results in telomeres of maximum length in the zygote.

Normal shortening of telomeres may protect organisms from cancer by limiting the number of divisions that somatic cells can undergo. Cells from large tumors often have unusually short telomeres, as we would expect for cells that have undergone many cell divisions. Further shortening would presumably lead to self-destruction of the tumor cells. Telomerase activity is abnormally high in cancerous somatic cells, suggesting that its ability to stabilize telomere length may allow these cancer cells to persist. Many cancer cells do seem capable of unlimited cell division, as do immortal strains of cultured cells (see Concept 12.3). For several years, researchers have studied inhibition of telomerase as a possible cancer therapy. While studies that inhibited telomerase in mice with tumors have led to the death of cancer cells, eventually the cells have restored the length of their telomeres by an alternative pathway. This is an area of ongoing research that may eventually yield useful cancer treatments.

CONCEPT CHECK 16.2

1. What role does complementary base pairing play in the replication of DNA?
2. How do immortal cells cope with the shortening of telomeres to be able to divide indefinitely?
3. **MAKE CONNECTIONS** ► What is the relationship between DNA replication and the S phase of the cell cycle? See Figure 12.6.
4. **VISUAL SKILLS** ► If the DNA pol I in a given cell were nonfunctional, how would that affect the synthesis of a *leading* strand? In the overview box in Figure 16.17, point out where DNA pol I would normally function on the top leading strand.

For suggested answers, see Appendix A.

CONCEPT 16.3

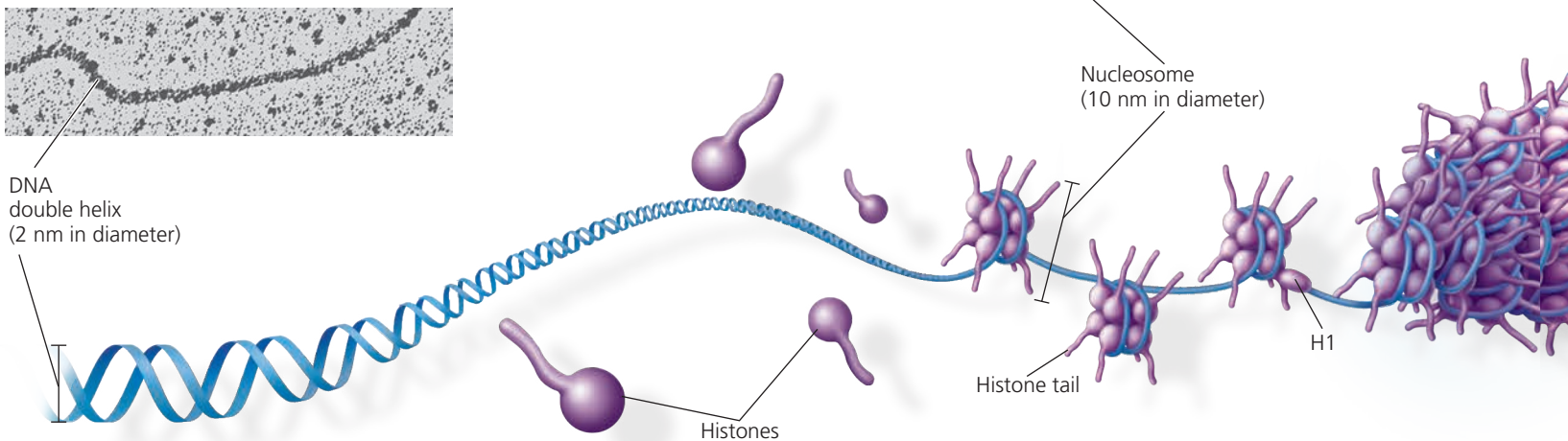
A chromosome consists of a DNA molecule packed together with proteins

Now that you have learned about the structure and replication of DNA, let's take a step back and examine how DNA is packaged into chromosomes, the structures that carry genetic information. The main component of the genome in most bacteria is one double-stranded, circular DNA

molecule that is associated with a small amount of protein. Although we refer to this structure as the bacterial chromosome, it is very different from a eukaryotic chromosome, which consists of one linear DNA molecule associated with a large amount of protein. In *E. coli*, the chromosomal DNA consists of about 4.6 million nucleotide pairs, representing about 4,400 genes. This is 100 times more DNA than is found in a typical virus, but only about one-thousandth as much DNA as in a human somatic cell. Still, that is a tremendous amount of DNA to be packaged in such a small container.

▼ Figure 16.22 Exploring Chromatin Packing in a Eukaryotic Chromosome

This illustration, accompanied by transmission electron micrographs, depicts a current model for the progressive levels of DNA coiling and folding. The illustration zooms out from a single molecule of DNA to a metaphase chromosome, which is large enough to be seen with a light microscope.



DNA, the double helix

Shown above is a ribbon model of DNA, with each ribbon representing one of the polynucleotide strands. Recall that each phosphate group along the backbone contributes a negative charge along the outside of each strand. The TEM shows a molecule of naked (protein-free) DNA; the double helix alone is 2 nm across.

Histones

Proteins called **histones** are responsible for the first level of DNA packing in chromatin. Although each histone is small—containing only about 100 amino acids—the total mass of histone in chromatin roughly equals the mass of DNA. More than a fifth of a histone's amino acids are positively charged (lysine or arginine) and therefore bind tightly to the negatively charged DNA.

Four types of histones are most common in chromatin. The histones are very similar among eukaryotes; for example, histones of the same type in cows and pea plants differ by only two amino acids. The apparent conservation of histone genes during evolution probably reflects the important role of histones in organizing DNA within cells.

These four types of histones are critical to the next level of DNA packing. (A fifth type of histone is involved in a further stage of packing.)

Nucleosomes, or “beads on a string” (10-nm fiber)

In electron micrographs, unfolded chromatin is 10 nm in diameter (the *10-nm fiber*). Such chromatin resembles beads on a string (see the TEM). Each “bead” is a **nucleosome**, the basic unit of DNA packing; the “string” between beads is called *linker DNA*.

A nucleosome consists of DNA wound twice around a protein core of eight histones, two each of the main histone types. The amino end (N-terminus) of each histone (the *histone tail*) extends outward from the nucleosome.

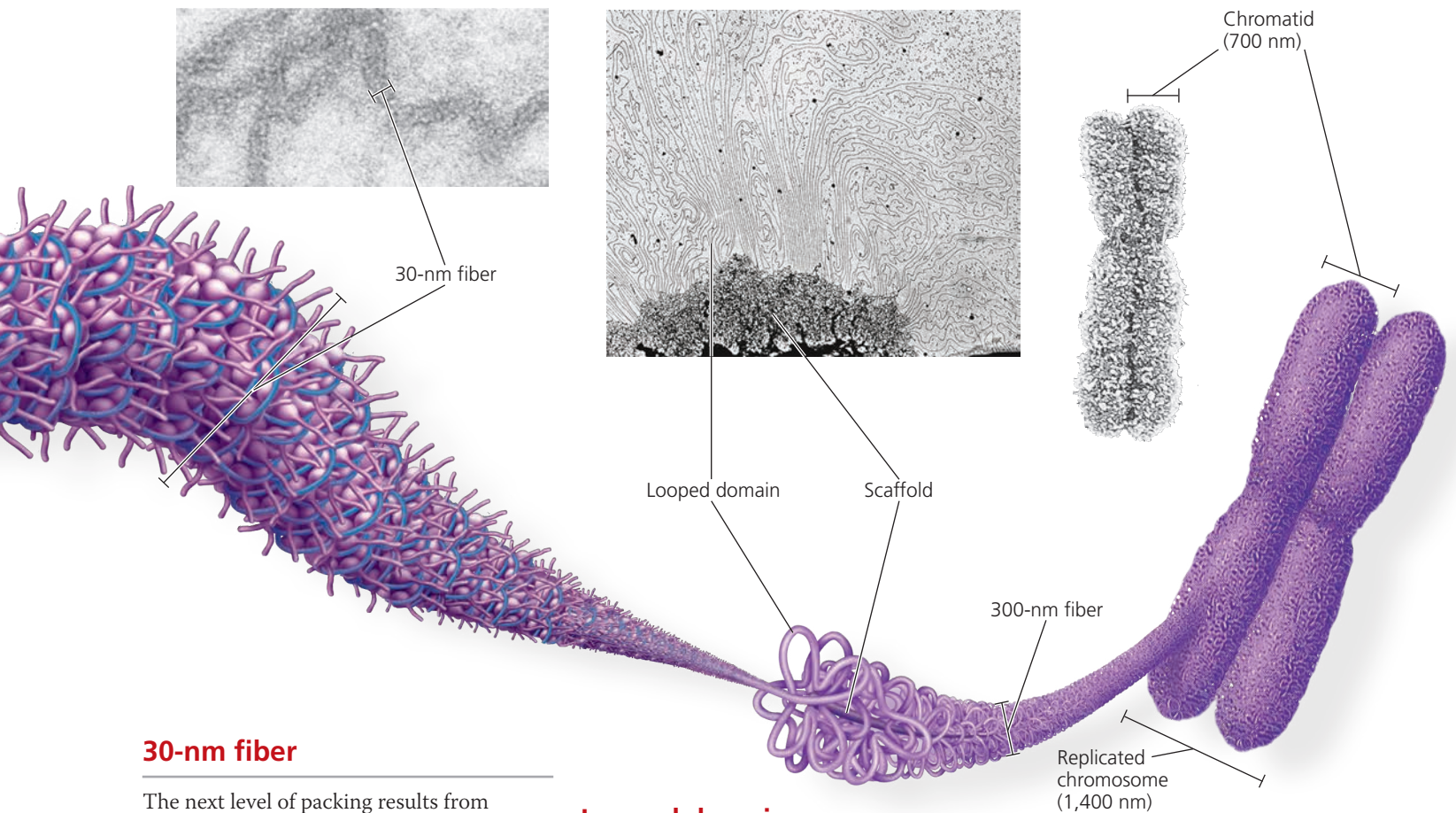
In the cell cycle, the histones leave the DNA only briefly during DNA replication. Generally, they do the same during the process of transcription, which also requires access to the DNA by the cell's molecular machinery. Nucleosomes, and in particular their histone tails, are involved in the regulation of gene expression.

Stretched out, the DNA of an *E. coli* cell would measure about a millimeter in length, which is 500 times longer than the cell. Within a bacterium, however, certain proteins cause the chromosome to coil and “supercoil,” densely packing it so that it fills only part of the cell. Unlike the nucleus of a eukaryotic cell, this dense region of DNA in a bacterium, called the nucleoid, is not bounded by membrane (see Figure 7.5).

Each eukaryotic chromosome contains a single linear DNA double helix that, in humans, averages about 1.5×10^8 nucleotide pairs. This is an enormous amount of DNA relative to a chromosome’s condensed length. If completely

stretched out, such a DNA molecule would be about 4 cm long, thousands of times the diameter of a cell nucleus—and that’s not even considering the DNA of the other 45 human chromosomes!

In the cell, eukaryotic DNA is precisely combined with a large amount of protein. Together, this complex of DNA and protein, called **chromatin**, fits into the nucleus through an elaborate, multilevel system of packing. Our current view of the successive levels of DNA packing in a chromosome is outlined in **Figure 16.22**. Study this figure carefully before reading further.



30-nm fiber

The next level of packing results from interactions between the histone tails of one nucleosome and the linker DNA and nucleosomes on either side. The fifth type of histone is involved at this level. These interactions cause the extended 10-nm fiber to coil or fold, forming a chromatin fiber roughly 30 nm in thickness, the *30-nm fiber*. Although the 30-nm fiber is quite prevalent in the interphase nucleus, the packing arrangement of nucleosomes in this form of chromatin is still a matter of some debate.

Looped domains (300-nm fiber)

The 30-nm fiber, in turn, forms loops called *looped domains* attached to a chromosome scaffold composed of proteins, thus making up a *300-nm fiber*. The scaffold is rich in one type of topoisomerase.

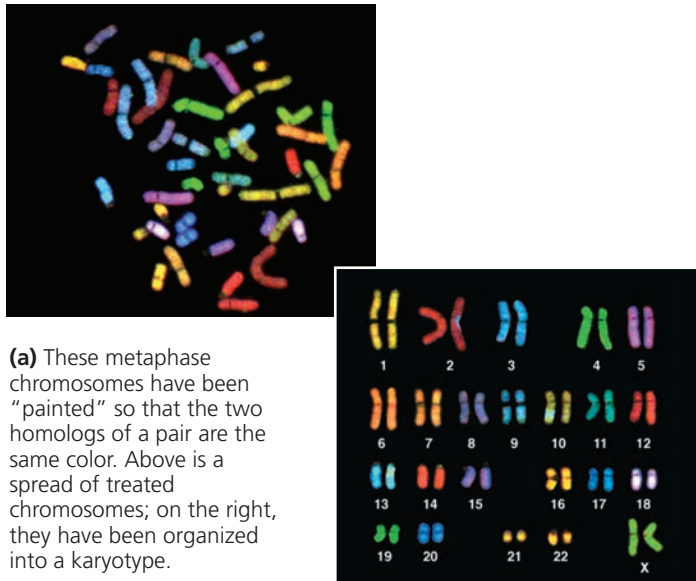
Metaphase chromosome

In a mitotic chromosome, the looped domains themselves coil and fold in a manner not yet fully understood, further compacting all the chromatin to produce the characteristic metaphase chromosome (also shown in the micrograph above). The width of one chromatid is 700 nm. Particular genes always end up located at the same places in metaphase chromosomes, indicating that the packing steps are highly specific and precise.

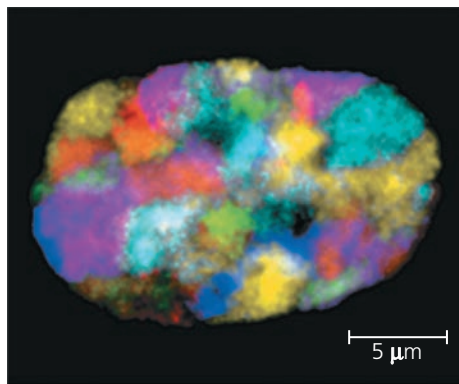
Chromatin undergoes striking changes in its degree of packing during the course of the cell cycle (see Figure 12.7). In interphase cells stained for light microscopy, the chromatin usually appears as a diffuse mass within the nucleus, suggesting that the chromatin is highly extended. As a cell prepares for mitosis, its chromatin coils and folds up (condenses), eventually forming a characteristic number of short, thick metaphase chromosomes that are distinguishable from each other with the light microscope (**Figure 16.23a**).

Though interphase chromatin is generally much less condensed than the chromatin of mitotic chromosomes, it shows several of the same levels of higher-order packing.

▼ **Figure 16.23 “Painting” chromosomes.** Researchers can treat (“paint”) human chromosomes with molecular tags that cause each chromosome pair to appear a different color.



(a) These metaphase chromosomes have been “painted” so that the two homologs of a pair are the same color. Above is a spread of treated chromosomes; on the right, they have been organized into a karyotype.



(b) The ability to visually distinguish among chromosomes makes it possible to see how the chromosomes are arranged in the interphase nucleus. Each chromosome appears to occupy a specific territory during interphase. In general, the two homologs of a pair are not located together.

MAKE CONNECTIONS ► If you arrested a human cell in metaphase I of meiosis and applied this technique, what would you observe? How would this differ from what you would see in metaphase of mitosis? Review Figure 13.8 and Figure 12.7.

Some of the chromatin comprising a chromosome seems to be present as a 10-nm fiber, but much is compacted into a 30-nm fiber, which in some regions is further folded into looped domains. Early on, biologists assumed that interphase chromatin was a tangled mass in the nucleus, like a bowl of spaghetti, but this is far from the case. Although an interphase chromosome lacks an obvious scaffold, its looped domains appear to be attached to the nuclear lamina, on the inside of the nuclear envelope, and perhaps also to fibers of the nuclear matrix. These attachments may help organize regions of chromatin where genes are active. The chromatin of each chromosome occupies a specific restricted area within the interphase nucleus, and the chromatin fibers of different chromosomes do not appear to be entangled (**Figure 16.23b**).

Even during interphase, the centromeres and telomeres of chromosomes, as well as other chromosomal regions in some cells, exist in a highly condensed state similar to that seen in a metaphase chromosome. This type of interphase chromatin, visible as irregular clumps with a light microscope, is called **heterochromatin**, to distinguish it from the less compacted, more dispersed **euchromatin** (“true chromatin”). Because of its compaction, heterochromatic DNA is largely inaccessible to the machinery in the cell responsible for transcribing the genetic information coded in the DNA, a crucial early step in gene expression. In contrast, the looser packing of euchromatin makes its DNA accessible to this machinery, so the genes present in euchromatin can be transcribed. The chromosome is a dynamic structure that is condensed, loosened, modified, and remodeled as necessary for various cell processes, including mitosis, meiosis, and gene activity. Chemical modifications of histones affect the state of chromatin condensation and also have multiple effects on gene activity, as you’ll see in Concept 18.2.

In this chapter, you have learned how DNA molecules are arranged in chromosomes and how DNA replication provides the copies of genes that parents pass to offspring. However, it is not enough that genes be copied and transmitted; the information they carry must be used by the cell. In other words, genes must also be expressed. In the next chapter, we will examine how the cell expresses the genetic information encoded in DNA.

CONCEPT CHECK 16.3

1. Describe the structure of a nucleosome, the basic unit of DNA packing in eukaryotic cells.
2. What two properties, one structural and one functional, distinguish heterochromatin from euchromatin?
3. **MAKE CONNECTIONS** ► Interphase chromosomes appear to be attached to the nuclear lamina and perhaps also the nuclear matrix. Describe these two structures. See Figure 7.9 and the associated text.

For suggested answers, see Appendix A.

16 Chapter Review



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SUMMARY OF KEY CONCEPTS

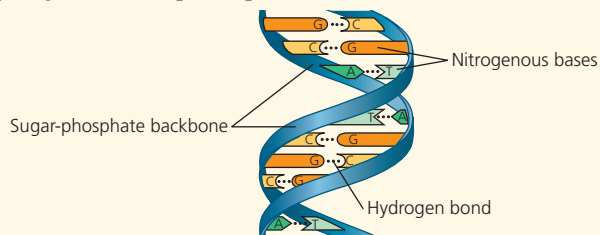
CONCEPT 16.1

DNA is the genetic material (pp. 365–370)

- Experiments with bacteria and with **phages** provided the first strong evidence that the genetic material is DNA.
- Watson and Crick deduced that DNA is a **double helix** and built a structural model. Two **antiparallel** sugar-phosphate chains wind around the outside of the molecule; the nitrogenous bases project into the interior, where they hydrogen-bond in specific pairs, A with T, G with C.



VOCAB
SELF-QUIZ
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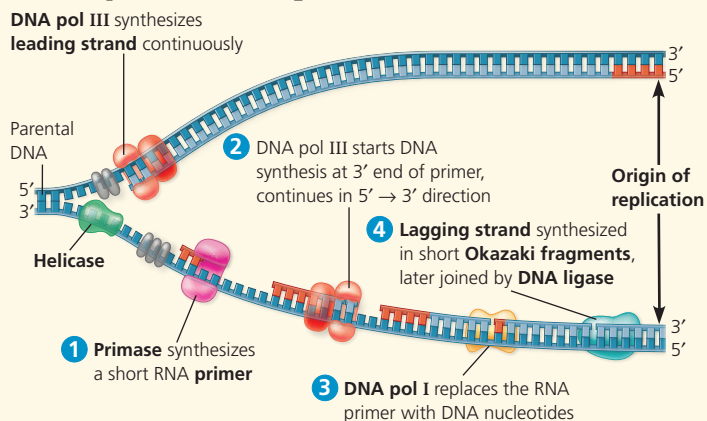


- ? What does it mean when we say that the two DNA strands in the double helix are antiparallel? What would an end of the double helix look like if the strands were parallel?

CONCEPT 16.2

Many proteins work together in DNA replication and repair (pp. 370–379)

- The Meselson-Stahl experiment showed that **DNA replication** is **semiconservative**: The parental molecule unwinds, and each strand then serves as a template for the synthesis of a new strand according to base-pairing rules.
- DNA replication at one **replication fork** is summarized here:



- DNA polymerases** proofread new DNA, replacing incorrect nucleotides. In **mismatch repair**, enzymes correct errors that persist. **Nucleotide excision repair** is a process by which **nucleases** cut out and other enzymes replace damaged stretches of DNA.
- The ends of eukaryotic chromosomal DNA get shorter with each round of replication. The presence of **telomeres**, repetitive sequences at the ends of linear DNA molecules, postpones the erosion of genes. Telomerase catalyzes the lengthening of telomeres in germ cells.

- ? Compare DNA replication on the leading and lagging strands, including both similarities and differences.

CONCEPT 16.3

A chromosome consists of a DNA molecule packed together with proteins (pp. 380–382)

- The chromosome of most bacterial species is a circular DNA molecule with some associated proteins, making up the nucleoid. The **chromatin** making up a eukaryotic chromosome is composed of DNA, **histones**, and other proteins. The histones bind to each other and to the DNA to form **nucleosomes**, the most basic units of DNA packing. Histone tails extend outward from each bead-like nucleosome core. Additional coiling and folding lead ultimately to the highly condensed chromatin of the metaphase chromosome.
- Chromosomes occupy restricted areas in the interphase nucleus. In interphase cells, most chromatin is less compacted (**euchromatin**), but some remains highly condensed (**heterochromatin**). Euchromatin, but not heterochromatin, is generally accessible for transcription of genes.

- ? Describe the levels of chromatin packing you'd expect to see in an interphase nucleus.

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–8 can be found in the Study Area in MasteringBiology.

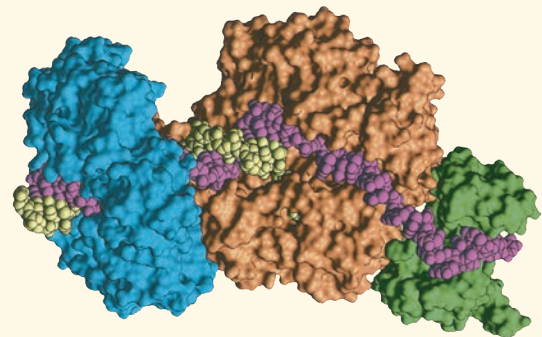
9. **MAKE CONNECTIONS** Although the proteins that cause the *E. coli* chromosome to coil are not histones, what property would you expect them to share with histones, given their ability to bind to DNA (see Figure 5.14)?



PRACTICE
TEST
goo.gl/iAsVgI

10. **EVOLUTION CONNECTION** Some bacteria may be able to respond to environmental stress by increasing the rate at which mutations occur during cell division. How might this be accomplished? Might there be an evolutionary advantage to this ability? Explain.

11. **SCIENTIFIC INQUIRY**

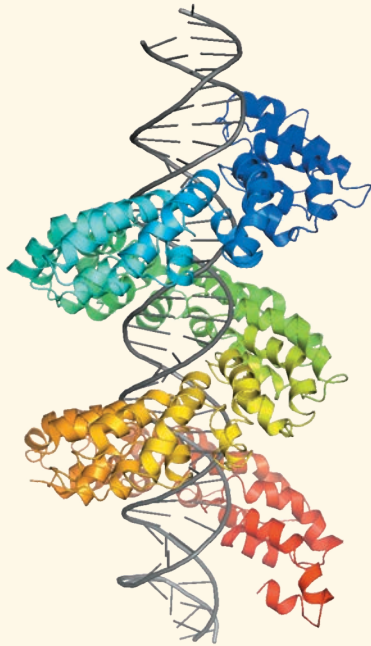


DRAW IT Model building can be an important part of the scientific process. The illustration shown above is a computer-generated model of a DNA replication complex. The parental and newly synthesized DNA strands are color-coded differently, as are each of the following three proteins: DNA pol III, the sliding clamp, and single-strand binding protein.

- Using what you've learned in this chapter to clarify this model, label each DNA strand and each protein.
- Draw an arrow to indicate the overall direction of DNA replication.

- 12. WRITE ABOUT A THEME: INFORMATION** The continuity of life is based on heritable information in the form of DNA, and structure and function are correlated at all levels of biological organization. In a short essay (100–150 words), describe how the structure of DNA is correlated with its role as the molecular basis of inheritance.

13. SYNTHESIZE YOUR KNOWLEDGE



This image shows DNA (gray) interacting with a computer-generated model of a TAL protein (multicolored), one of a family of proteins found only in a species of the bacterium *Xanthomonas*. The bacterium uses proteins like this one to find specific gene sequences in cells of the organisms it infects, such as tomatoes, rice, and citrus fruits. Given what you know about DNA structure and considering the image above, discuss how the TAL protein's structure suggests that it functions.

For selected answers, see Appendix A.



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Expression of Genes

17



▲ **Figure 17.1** How does a single faulty gene result in the dramatic appearance of these albino donkeys?

KEY CONCEPTS

- 17.1** Genes specify proteins via transcription and translation
- 17.2** Transcription is the DNA-directed synthesis of RNA: *a closer look*
- 17.3** Eukaryotic cells modify RNA after transcription
- 17.4** Translation is the RNA-directed synthesis of a polypeptide: *a closer look*
- 17.5** Mutations of one or a few nucleotides can affect protein structure and function

▼ **An albino raccoon.**



The Flow of Genetic Information

The island of Asinara lies off the coast of the Italian island of Sardinia. The name Asinara probably originated from the Latin word *sinuaria*, which means “sinus-shaped.” A second meaning of Asinara is “donkey-inhabited,” which is perhaps even more appropriate because Asinara is home to a wild population of albino donkeys (**Figure 17.1**). What factors are responsible for the albino phenotype?

Inherited traits are determined by genes, and the trait of albinism is caused by a recessive allele of a pigmentation gene (see Concept 14.4). The information content of genes is in the form of specific sequences of nucleotides along strands of DNA, the genetic material. The albino donkey has a faulty version of a key protein, an enzyme required for pigment synthesis, and this protein is faulty because the gene that codes for it contains incorrect information.

This example illustrates the main point of this chapter: The DNA inherited by an organism leads to specific traits by dictating the synthesis of proteins and of RNA molecules involved in protein synthesis. In other words, proteins are the link between genotype and phenotype. **Gene expression** is the process by which DNA directs the synthesis of proteins (or, in some cases, just RNAs). The expression of genes that code for proteins includes two stages: transcription and translation. This chapter describes the flow of information from gene to protein and explains how genetic mutations affect organisms through their proteins. Understanding the processes of gene expression will allow us to revisit the concept of the gene in more detail at the end of the chapter.

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Get Ready for This Chapter

CONCEPT 17.1

Genes specify proteins via transcription and translation

Before going into the details of how genes direct protein synthesis, let's step back and examine how the fundamental relationship between genes and proteins was discovered.

Evidence from Studying Metabolic Defects

In 1902, British physician Archibald Garrod was the first to suggest that genes dictate phenotypes through enzymes, proteins that catalyze specific chemical reactions in the cell. He postulated that the symptoms of an inherited disease reflect an inability to make a particular enzyme. He later referred to such diseases as “inborn errors of metabolism.” For example, people with a disease called alkaptonuria have black urine because it contains a chemical called alkapton, which darkens upon exposure to air. Garrod reasoned that most people have an enzyme that breaks down alkapton, whereas people with alkaptonuria have inherited an inability to make that enzyme, so alkapton is expelled in their urine.

Several decades later, research supported Garrod's hypothesis that a gene dictates the production of a specific enzyme, later named the *one gene–one enzyme hypothesis*. Biochemists learned that cells synthesize and degrade most organic molecules via metabolic pathways, in which each chemical reaction in a sequence is catalyzed by a specific enzyme (see Concept 6.1). Such metabolic pathways lead, for instance, to the synthesis of the pigments that give the brown donkey in Figure 17.1 its fur color or fruit flies (*Drosophila*) their eye color (see Figure 15.3). In the 1930s, the American biochemist and geneticist George Beadle and his French colleague Boris Ephrussi speculated that in *Drosophila*, each mutation affecting eye color blocks pigment synthesis at a specific step by preventing production of the enzyme that catalyzes that step. But neither the chemical reactions nor the enzymes that catalyze them were known at the time.

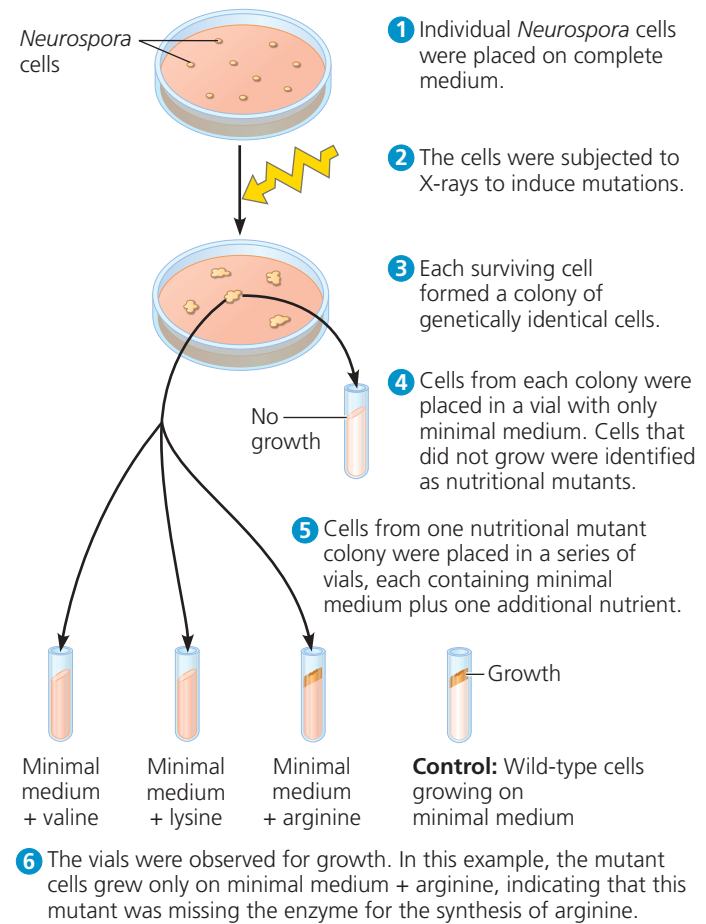
Nutritional Mutants in *Neurospora*: Scientific Inquiry

A breakthrough came a few years later at Stanford University, where Beadle and Edward Tatum began working with a bread mold, *Neurospora crassa*, a haploid species. To observe a change in a mutant's phenotype, Beadle and Tatum needed to disable just one allele (rather than two, as in a diploid species) of a protein-coding gene required for a specific metabolic activity. They bombarded *Neurospora* with X-rays, known to cause mutations, and looked among the survivors for mutants that differed in their nutritional needs from the wild-type bread mold.

Wild-type *Neurospora* has modest food requirements. It can grow in the laboratory on a simple solution containing

▼ Figure 17.2 Beadle and Tatum's experimental approach.

To obtain nutritional mutants, Beadle and Tatum exposed *Neurospora* cells to X-rays, inducing mutations, then screened mutants that had new nutritional requirements, such as arginine, as shown here.



minimal nutrients for growth—inorganic salts, glucose, and the vitamin biotin—incorporated into agar, a support medium. From this so-called *minimal medium*, wild-type mold cells use their metabolic pathways to produce all the other molecules they need for growth, dividing repeatedly and forming visible colonies of genetically identical cells. As shown in Figure 17.2, Beadle and Tatum generated different “nutritional mutants” of *Neurospora* cells, each of which was unable to synthesize a particular essential nutrient. Such cells could not grow on minimal medium but could grow on *complete medium*, which contains all nutrients needed for growth. For *Neurospora*, the complete medium consists of the minimal medium supplemented with all 20 amino acids and a few other nutrients. Beadle and Tatum hypothesized that in each nutritional mutant, the gene for the enzyme that synthesizes a particular nutrient had been disabled.

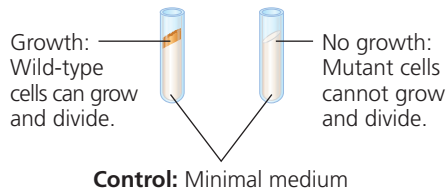
This approach resulted in a valuable collection of mutant strains of *Neurospora*, catalogued by their defect in a particular pathway. Two colleagues of theirs, Adrian Srb and Norman Horowitz, used a collection of arginine-requiring mutants to investigate the biochemical pathway for arginine synthesis in *Neurospora* (Figure 17.3). Srb and Horowitz pinned down each

▼ Figure 17.3

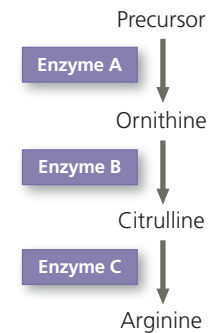
Inquiry Do individual genes specify the enzymes that function in a biochemical pathway?

Experiment Working with the mold *Neurospora crassa*, Adrian Srb and Norman Horowitz, then at Stanford University, used Beadle and Tatum's experimental approach (see Figure 17.2) to isolate mutants that required arginine in their growth medium. The researchers showed that these mutants fell into three classes, each defective in a different gene. From studies by others on mammalian liver cells, they suspected that the metabolic pathway of arginine biosynthesis involved a precursor nutrient and the intermediate molecules ornithine and citrulline, as shown in the diagram on the right.

Their most famous experiment, shown here, tested both the *one gene–one enzyme hypothesis* and their postulated arginine-synthesizing pathway. In this experiment, they grew their three classes of mutants under the four different conditions shown in the Results Table below. They included minimal medium (MM) as a control, knowing that wild-type cells could grow on MM but mutant cells could not. (See test tubes below.)



Results As shown in the table on the right, the wild-type strain was capable of growth under all experimental conditions, requiring only the minimal medium. The three classes of mutants each had a specific set of growth requirements. For example, class II mutants could not grow when ornithine alone was added but could grow when either citrulline or arginine was added.



Results Table					
Classes of <i>Neurospora crassa</i>					
Condition		Wild type	Class I mutants	Class II mutants	Class III mutants
	Minimal medium (MM) (control)				
	MM + ornithine				
	MM + citrulline				
	MM + arginine (control)				
Summary of results		Can grow with or without any supplements	Can grow on ornithine, citrulline, or arginine	Can grow only on citrulline or arginine	Require arginine to grow

Conclusion From the growth requirements of the mutants, Srb and Horowitz deduced that each class of mutant was unable to carry out one step in the pathway for synthesizing arginine, presumably because it lacked the necessary enzyme, as shown in the table on the right. Because each of their mutants was mutated in a single gene, they concluded that each mutated gene must normally dictate the production of one enzyme. Their results supported the one gene–one enzyme hypothesis, proposed by Beadle and Tatum, and also confirmed that the arginine pathway described in the mammalian liver also operates in *Neurospora*. (Notice in the Results Table that a mutant can grow only if supplied with a compound made *after* the defective step because this bypasses the defect.)

Data from A. M. Srb and N. H. Horowitz, The ornithine cycle in *Neurospora* and its genetic control, *Journal of Biological Chemistry* 154:129–139 (1944).

Gene (codes for enzyme)	Wild type	Class I mutants (mutation in gene A)	Class II mutants (mutation in gene B)	Class III mutants (mutation in gene C)
Gene A	Precursor → Enzyme A → Ornithine	~~Precursor → Enzyme A~~ → Ornithine	Precursor → Enzyme A → Ornithine	Precursor → Enzyme A → Ornithine
Gene B	Ornithine → Enzyme B → Citrulline	Ornithine → Enzyme B → Citrulline	~~Ornithine → Enzyme B~~ → Citrulline	Ornithine → Enzyme B → Citrulline
Gene C	Citrulline → Enzyme C → Arginine	Citrulline → Enzyme C → Arginine	Citrulline → Enzyme C → Arginine	~~Citrulline → Enzyme C~~ → Arginine

WHAT IF? > Suppose the experiment had shown that class I mutants could grow only in MM supplemented by ornithine or arginine and that class II mutants could grow in MM supplemented by citrulline, ornithine, or arginine. What conclusions would the researchers have drawn from those results regarding the biochemical pathway and the defect in class I and class II mutants?

mutant's defect more specifically, using additional tests to distinguish among three classes of arginine-requiring mutants. Mutants in each class required a different set of compounds along the arginine-synthesizing pathway, which has three steps. These results, and those of many similar experiments done by Beadle and Tatum, suggested that each class was blocked at a different step in this pathway because mutants in that class lacked the enzyme that catalyzes the blocked step.

Because Beadle and Tatum set up their experimental conditions so that each mutant was defective in a single gene, the collected results, taken together, provided strong support for a working hypothesis they had proposed earlier. The *one gene–one enzyme hypothesis*, as they dubbed it, states that the function of a gene is to dictate the production of a specific enzyme. Further support for this hypothesis came from experiments that identified the specific enzymes lacking in the mutants. Beadle and Tatum shared a Nobel Prize in 1958 for “their discovery that genes act by regulating definite chemical events” (in the words of the Nobel committee).

Today, we know of countless examples in which a mutation in a gene causes a faulty enzyme that in turn leads to an identifiable condition. The albino donkey in Figure 17.1 lacks a key enzyme called tyrosinase in the metabolic pathway that produces melanin, a dark pigment. The absence of melanin causes white fur and other effects throughout the donkey's body. Its nose, ears, and hooves, as well as its eyes, are pink because no melanin is present to mask the reddish color of the blood vessels that run through those structures.

The Products of Gene Expression: A Developing Story

As researchers learned more about proteins, they made revisions to the one gene–one enzyme hypothesis. First of all, not all proteins are enzymes. Keratin, the structural protein of animal hair, and the hormone insulin are two examples of nonenzyme proteins. Because proteins that are not enzymes are nevertheless gene products, molecular biologists began to think in terms of one gene–one protein. However, many proteins are constructed from two or more different polypeptide chains, and each polypeptide is specified by its own gene. For example, hemoglobin—the oxygen-transporting protein of vertebrate red blood cells—contains two kinds of polypeptides (see Figure 5.18), and thus two genes code for this protein, one for each type of polypeptide. Beadle and Tatum's idea was therefore restated as the *one gene–one polypeptide hypothesis*. Even this description is not entirely accurate, though. First, in many cases, a eukaryotic gene can code for a set of closely related polypeptides via a process called alternative splicing, which you will learn about later in this chapter. Second, quite a few genes code for RNA molecules that have important functions in

cells even though they are never translated into protein. For now, we will focus on genes that do code for polypeptides. (Note that it is common to refer to these gene products as proteins—a practice you will encounter in this book—rather than more precisely as polypeptides.)

Basic Principles of Transcription and Translation

Genes provide the instructions for making specific proteins. But a gene does not build a protein directly. The bridge between DNA and protein synthesis is the nucleic acid RNA. RNA is chemically similar to DNA except that it contains ribose instead of deoxyribose as its sugar and has the nitrogenous base uracil rather than thymine (see Figure 5.23). Thus, each nucleotide along a DNA strand has A, G, C, or T as its base, and each nucleotide along an RNA strand has A, G, C, or U as its base. An RNA molecule usually consists of a single strand.

It is customary to describe the flow of information from gene to protein in linguistic terms. Just as specific sequences of letters communicate information in a language such as English, both nucleic acids and proteins are polymers with specific sequences of monomers that convey information. In DNA or RNA, the monomers are the four types of nucleotides, which differ in their nitrogenous bases. Genes are typically hundreds or thousands of nucleotides long, each gene having a specific sequence of nucleotides. Each polypeptide of a protein also has monomers arranged in a particular linear order (the protein's primary structure; see Figure 5.18), but its monomers are amino acids. Thus, nucleic acids and proteins contain information written in two different chemical languages. Getting from DNA to protein requires two major stages: transcription and translation.

Transcription is the synthesis of RNA using information in the DNA. The two nucleic acids are written in different forms of the same language, and the information is simply transcribed, or “rewritten,” from DNA to RNA. Just as a DNA strand provides a template for making a new complementary strand during DNA replication (see Concept 16.2), it also can serve as a template for assembling a complementary sequence of RNA nucleotides. For a protein-coding gene, the resulting RNA molecule is a faithful transcript of the gene's protein-building instructions. This type of RNA molecule is called **messenger RNA (mRNA)** because it carries a genetic message from the DNA to the protein-synthesizing machinery of the cell. (Transcription is the general term for the synthesis of *any* kind of RNA on a DNA template. Later, you will learn about some other types of RNA produced by transcription.)

Translation is the synthesis of a polypeptide using the information in the mRNA. During this stage, there is a change in language: The cell must translate the nucleotide sequence of an mRNA molecule into the amino acid sequence of

a polypeptide. The sites of translation are **ribosomes**, molecular complexes that facilitate the orderly linking of amino acids into polypeptide chains.

Transcription and translation occur in all organisms. Because most studies have involved bacteria and eukaryotic cells, they are our main focus in this chapter. Our understanding of transcription and translation in archaea lags behind, but we do know that archaeal cells share some features of gene expression with bacteria and others with eukaryotes.

The basic mechanics of transcription and translation are similar for bacteria and eukaryotes, but there is an important difference in the flow of genetic information within the cells. Bacteria do not have nuclei. Therefore, nuclear membranes do not separate bacterial DNA and mRNA from ribosomes and the other protein-synthesizing equipment (**Figure 17.4a**). As you'll see later, this lack of compartmentalization allows translation of an mRNA to begin while its transcription is still in progress. By contrast, eukaryotic cells have nuclei. The presence of a nuclear envelope separates transcription from translation in space and time (**Figure 17.4b**). Transcription occurs in the nucleus, but the mRNA must be transported to the cytoplasm for translation. Before eukaryotic RNA transcripts from protein-coding genes can leave the nucleus, they are modified in various ways to produce the final, functional mRNA. The transcription of a protein-coding eukaryotic gene results in *pre-mRNA*, and further processing yields the finished mRNA. The initial RNA transcript from any gene, including those specifying RNA that is not translated into protein, is more generally called a **primary transcript**.

To summarize: Genes program protein synthesis via genetic messages in the form of messenger RNA. Put another way, cells are governed by a molecular chain of command with a directional flow of genetic information:

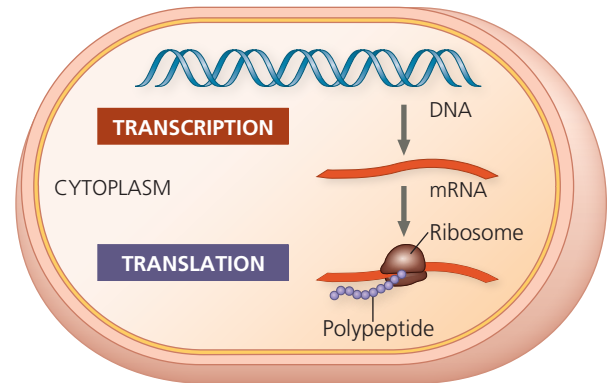


This concept was dubbed the *central dogma* by Francis Crick in 1956. But in the 1970s, scientists were surprised to discover some enzymes that use RNA molecules as templates for DNA synthesis (which we'll cover in Concept 26.2). However, these exceptions do not invalidate the idea that, in general, genetic information flows from DNA to RNA to protein. In the next section, we discuss how the instructions for assembling amino acids into a specific order are encoded in nucleic acids.

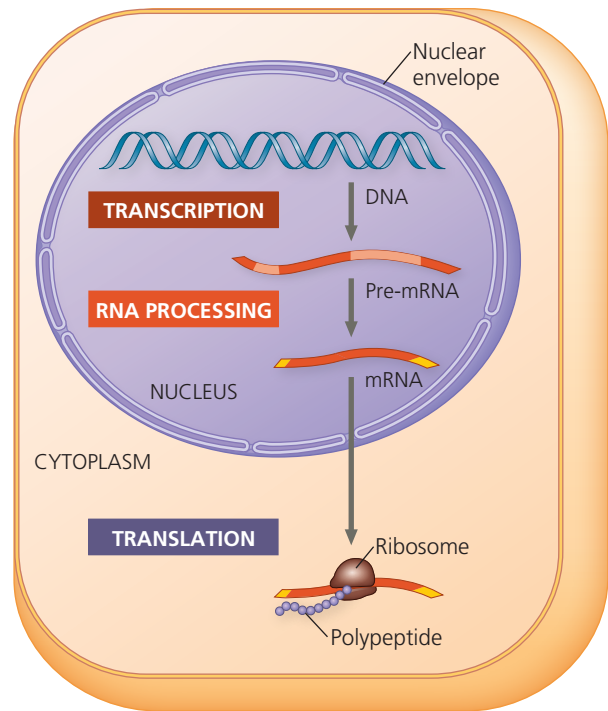
The Genetic Code

When biologists began to suspect that the instructions for protein synthesis were encoded in DNA, they recognized a problem: There are only four nucleotide bases to specify

▼ **Figure 17.4 Overview: the roles of transcription and translation in the flow of genetic information.** In a cell, inherited information flows from DNA to RNA to protein. The two main stages of information flow are transcription and translation. A miniature version of part (a) or (b) accompanies several figures later in the chapter as an orientation diagram to help you see where a particular figure fits into the overall scheme of gene expression.



(a) Bacterial cell. In a bacterial cell, which lacks a nucleus, mRNA produced by transcription is immediately translated without additional processing.



(b) Eukaryotic cell. The nucleus provides a separate compartment for transcription. The original RNA transcript, called pre-mRNA, is processed in various ways before leaving the nucleus as mRNA.

 **Animation: Overview of Protein Synthesis in Bacteria**
Animation: Overview of Protein Synthesis in Eukaryotes

20 amino acids. Thus, the genetic code cannot be a language like Chinese, where each written symbol corresponds to a word. How many nucleotides, then, would turn out to correspond to an amino acid?

Codons: Triplets of Nucleotides

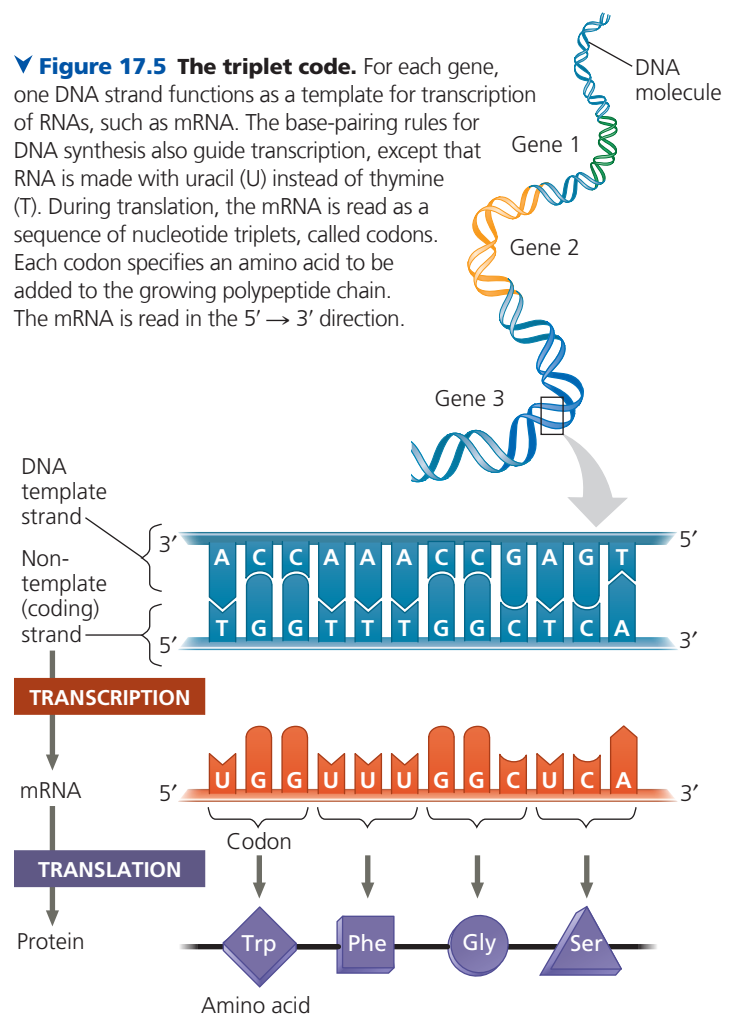
If each kind of nucleotide base were translated into an amino acid, only four amino acids could be specified, one per nucleotide base. Would a language of two-letter code words suffice? The two-nucleotide sequence AG, for example, could specify one amino acid, and GT could specify another. Since there are four possible nucleotide bases in each position, this would give us 16 (that is, 4×4 , or 4^2) possible arrangements—still not enough to code for all 20 amino acids.

Triplets of nucleotide bases are the smallest units of uniform length that can code for all the amino acids. If each arrangement of three consecutive nucleotide bases specifies an amino acid, there can be 64 (that is, 4^3) possible code words—more than enough to specify all the amino acids. Experiments have verified that the flow of information from gene to protein is based on a **triplet code**: The genetic instructions for a polypeptide chain are written in the DNA as a series of nonoverlapping, three-nucleotide words. The series of words in a gene is transcribed into a complementary series of nonoverlapping, three-nucleotide words in mRNA, which is then translated into a chain of amino acids (Figure 17.5).

During transcription, the gene determines the sequence of nucleotide bases along the length of the RNA molecule that is being synthesized. For each gene, only one of the two DNA strands is transcribed. This strand is called the **template strand** because it provides the pattern, or template, for the sequence of nucleotides in an RNA transcript. For any given gene, the same strand is used as the template every time the gene is transcribed. However, farther along on the same chromosomal DNA molecule, the opposite strand may function as the template for a different gene. The strand that is used as the template is determined by the orientation of the enzyme that transcribes genes, which in turn depends on the particular DNA sequences associated with that gene.

An mRNA molecule is complementary rather than identical to its DNA template because RNA nucleotides are assembled on the template according to base-pairing rules (see Figure 17.5). The pairs are similar to those that form during DNA replication, except that U (the RNA substitute for T) pairs with A and the mRNA nucleotides contain ribose instead of deoxyribose. Like a new strand of DNA, the RNA molecule is synthesized in an antiparallel direction to the template strand of DNA. (To review what is meant by “antiparallel” and the 5′ and 3′ ends of a nucleic acid chain, see Figure 16.7.) In the example in Figure 17.5, the nucleotide triplet ACC along the DNA template strand (written as 3′-ACC-5′) provides a template for 5′-UGG-3′ in the mRNA molecule. The mRNA nucleotide triplets are called **codons**, and they are customarily written in the 5′ → 3′ direction. In our example, UGG is the codon for the amino acid tryptophan (abbreviated Trp, or W). The term *codon* is also used

▼ **Figure 17.5 The triplet code.** For each gene, one DNA strand functions as a template for transcription of RNAs, such as mRNA. The base-pairing rules for DNA synthesis also guide transcription, except that RNA is made with uracil (U) instead of thymine (T). During translation, the mRNA is read as a sequence of nucleotide triplets, called codons. Each codon specifies an amino acid to be added to the growing polypeptide chain. The mRNA is read in the 5′ → 3′ direction.



VISUAL SKILLS ► By convention, the nontemplate strand, also called the coding strand, is used to represent a DNA sequence. Write the sequence of the mRNA strand and the nontemplate strand—in both cases reading from 5′ to 3′—and compare them. Why do you think this convention was adopted? (Hint: Why is this called the coding strand?)

MP3 Tutor: DNA to RNA to Protein

for the DNA nucleotide triplets along the *nontemplate* strand. These codons are complementary to the template strand and thus identical in sequence to the mRNA, except that they have a T wherever there is a U in the mRNA. For this reason, the nontemplate DNA strand is often called the **coding strand**; by convention, the sequence of the coding strand is used when a gene’s sequence is reported.

During translation, the sequence of codons along an mRNA molecule is decoded, or translated, into a sequence of amino acids making up a polypeptide chain. The codons are read by the translation machinery in the 5′ → 3′ direction along the mRNA. Each codon specifies which one of the 20 amino acids will be incorporated at the corresponding position along a polypeptide. Because codons are nucleotide triplets, the number of nucleotides making up a genetic message must be three times the number of amino acids in the protein product. For example, it takes 300 nucleotides along an mRNA strand to code for the amino acids in a polypeptide that is 100 amino acids long.

Cracking the Code

Molecular biologists cracked the genetic code of life in the early 1960s when a series of elegant experiments disclosed the amino acid translations of each of the RNA codons. The first codon was deciphered in 1961 by Marshall Nirenberg, of the National Institutes of Health, along with his colleagues. Nirenberg synthesized an artificial mRNA by linking together many identical RNA nucleotides containing uracil as their base. No matter where the genetic message started or stopped, it could contain only one codon (UUU) over and over. Nirenberg added this “poly-U” polynucleotide to a test-tube mixture containing amino acids, ribosomes, and the other components required for protein synthesis. His artificial system translated the poly-U mRNA into a polypeptide containing many units of the amino acid phenylalanine (Phe, or F), strung together as a long polyphenylalanine chain. Thus, Nirenberg determined that the mRNA codon UUU specifies the amino acid phenylalanine. Soon, the amino acids specified by the codons AAA, GGG, and CCC were also identified.

Although more elaborate techniques were required to decode mixed triplets such as AUA and CGA, all 64 codons were deciphered by the mid-1960s. As **Figure 17.6** shows, 61 of the 64 triplets code for amino acids. The three codons that do not designate amino acids are “stop” signals, or termination codons, marking the end of translation. Notice that the codon AUG has a dual function: It codes for the amino acid methionine (Met, or M) and also functions as a “start” signal, or initiation codon. Genetic messages usually begin with the mRNA codon AUG, which signals the protein-synthesizing machinery to begin translating the mRNA at that location. (Because AUG also stands for methionine, polypeptide chains begin with methionine when they are synthesized. However, an enzyme may subsequently remove this starter amino acid from the chain.)

Notice in **Figure 17.6** that there is redundancy in the genetic code, but no ambiguity. For example, although codons GAA and GAG both specify glutamic acid (redundancy), neither of them ever specifies any other amino acid (no ambiguity). The redundancy in the code is not altogether random. In many cases, codons that are synonyms for a particular amino acid differ only in the third nucleotide base of the triplet. We will consider the significance of this redundancy later in the chapter.

Our ability to extract the intended message from a written language depends on reading the symbols in the correct groupings—that is, in the correct **reading frame**. Consider this statement: “The red dog ate the bug.” Group the letters incorrectly by starting at the wrong point, and the result will probably be gibberish: for example, “her edd oga tet heb ug.” The reading frame is also important in the molecular language of cells. The short stretch of polypeptide shown in **Figure 17.5**, for instance, will be made

▼ **Figure 17.6 The codon table for mRNA.** The three nucleotide bases of an mRNA codon are designated here as the first, second, and third bases, reading in the 5′ → 3′ direction along the mRNA. The codon AUG not only stands for the amino acid methionine (Met, or M) but also functions as a “start” signal for ribosomes to begin translating the mRNA at that point. Three of the 64 codons function as “stop” signals, marking where ribosomes end translation. Both one- and three-letter codes are shown for the amino acids; see **Figure 5.14** for their full names.

		Second mRNA base				
		U	C	A	G	
First mRNA base (5' end of codon)	U	UUU } Phe (F) UUC } UUA } Leu (L) UUG }	UCU } UCC } Ser (S) UCA } UCG }	UAU } Tyr (Y) UAC } UAA Stop UAG Stop	UGU } Cys (C) UGC } UGA Stop UGG Trp (W)	U C A G
	C	CUU } CUC } Leu (L) CUA } CUG }	CCU } CCC } Pro (P) CCA } CCG }	CAU } His (H) CAC } CAA } Gln (Q) CAG }	CGU } CGC } Arg (R) CGA } CGG }	U C A G
	A	AUU } Ile (I) AUC } AUA } AUG Met (M) or start	ACU } ACC } Thr (T) ACA } ACG }	AAU } Asn (N) AAC } AAA } Lys (K) AAG }	AGU } Ser (S) AGC } AGA } Arg (R) AGG }	U C A G
	G	GUU } GUC } Val (V) GUA } GUG }	GCU } GCC } Ala (A) GCA } GCG }	GAU } Asp (D) GAC } GAA } Glu (E) GAG }	GGU } GGC } Gly (G) GGA } GGG }	U C A G
						Third mRNA base (3' end of codon)

VISUAL SKILLS ► A segment in the middle of an mRNA has the sequence 5'-AGAGAACCGCGA-3'. Using the codon table, translate this sequence, assuming the first three nucleotides are a codon.



Animation: Translation: The Genetic Code

correctly only if the mRNA nucleotides are read from left to right (5′ → 3′) in the groups of three shown in the figure: UGG UUU GGC UCA. Although a genetic message is written with no spaces between the codons, the cell’s protein-synthesizing machinery reads the message as a series of nonoverlapping three-letter words. The message is *not* read as a series of overlapping words—UGGUUU, and so on—which would convey a very different message.

Evolution of the Genetic Code

EVOLUTION The genetic code is nearly universal, shared by organisms from the simplest bacteria to the most complex plants and animals. The mRNA codon CCG, for instance, is translated as the amino acid proline in all organisms whose genetic code has been examined. In laboratory experiments, genes can be transcribed and translated after being transplanted from one species to another, sometimes with quite

▼ **Figure 17.7 Evidence for evolution: expression of genes from different species.** Because diverse forms of life share a common genetic code due to their shared ancestry, one species can be programmed to produce proteins characteristic of a second species by introducing DNA from the second species into the first.



(a) **Tobacco plant expressing a firefly gene.** The yellow glow is produced by a chemical reaction catalyzed by the protein product of the firefly gene.



(b) **Pig expressing a jellyfish gene.** Researchers injected a jellyfish gene for a fluorescent protein into fertilized pig eggs. One developed into this fluorescent pig.

 **Video: GFP Transgenic Mice**

striking results, as shown in **Figure 17.7**. Bacteria can be programmed by the insertion of human genes to synthesize certain human proteins for medical use, such as insulin. Such applications have produced many exciting developments in the area of biotechnology (see Concept 19.4).

Despite a small number of exceptions, the evolutionary significance of the code's near universality is clear. A language shared by all living things must have been operating very early in the history of life—early enough to be present in the common ancestor of all present-day organisms. A shared genetic vocabulary is a reminder of the kinship of all life.

CONCEPT CHECK 17.1

1. **MAKE CONNECTIONS** > In a research article about alkaptonuria published in 1902, Garrod suggested that humans inherit two “characters” (alleles) for a particular enzyme and that both parents must contribute a faulty version for the offspring to have alkaptonuria. Today, would this disorder be called dominant or recessive? (See Concept 14.4.)
2. Since AUG is the start codon and codes for methionine, do all proteins have methionine as the first amino acid? Explain.
3. **DRAW IT** > T The template strand of a gene contains the sequence 3'-TTCAGTCGT-5'. Imagine that the nontemplate sequence was transcribed instead of the template sequence. Draw the mRNA sequence and translate it using Figure 17.6. (Be sure to pay attention to the 5' and 3' ends.) Predict how well the protein synthesized from the nontemplate strand would function, if at all.

For suggested answers, see Appendix A.

CONCEPT 17.2

Transcription is the DNA-directed synthesis of RNA: a closer look

Now that we have considered the linguistic logic and evolutionary significance of the genetic code, we are ready to reexamine transcription, the first stage of gene expression, in greater detail.

Molecular Components of Transcription

Messenger RNA, the carrier of information from DNA to the cell's protein-synthesizing machinery, is transcribed from the template strand of a gene. An enzyme called an **RNA polymerase** pries the two strands of DNA apart and joins together RNA nucleotides complementary to the DNA template strand, thus elongating the RNA polynucleotide (**Figure 17.8**). Like the DNA polymerases that function in DNA replication, RNA polymerases can assemble a polynucleotide only in its 5' → 3' direction, adding onto its 3' end. Unlike DNA polymerases, however, RNA polymerases are able to start a chain from scratch; they don't need to add the first nucleotide onto a pre-existing primer.

Specific sequences of nucleotides along the DNA mark where transcription of a gene begins and ends. The DNA sequence where RNA polymerase attaches and initiates transcription is known as the **promoter**; in bacteria, the sequence that signals the end of transcription is called the **terminator**. (The termination mechanism is different in eukaryotes; we'll describe it later.) Molecular biologists refer to the direction of transcription as “downstream” and the other direction as “upstream.” These terms are also used to describe the positions of nucleotide sequences within the DNA or RNA. Thus, the promoter sequence in DNA is said to be upstream from the terminator. The stretch of DNA downstream from the promoter that is transcribed into an RNA molecule is called a **transcription unit**.

Bacteria have a single type of RNA polymerase that synthesizes not only mRNA but also other types of RNA that function in protein synthesis, such as ribosomal RNA. In contrast, eukaryotes have at least three types of RNA polymerase in their nuclei; the one used for pre-mRNA synthesis is called RNA polymerase II. The other RNA polymerases transcribe RNA molecules that are not translated into protein. In the discussion that follows, we start with the features of mRNA synthesis common to both bacteria and eukaryotes and then describe some key differences.

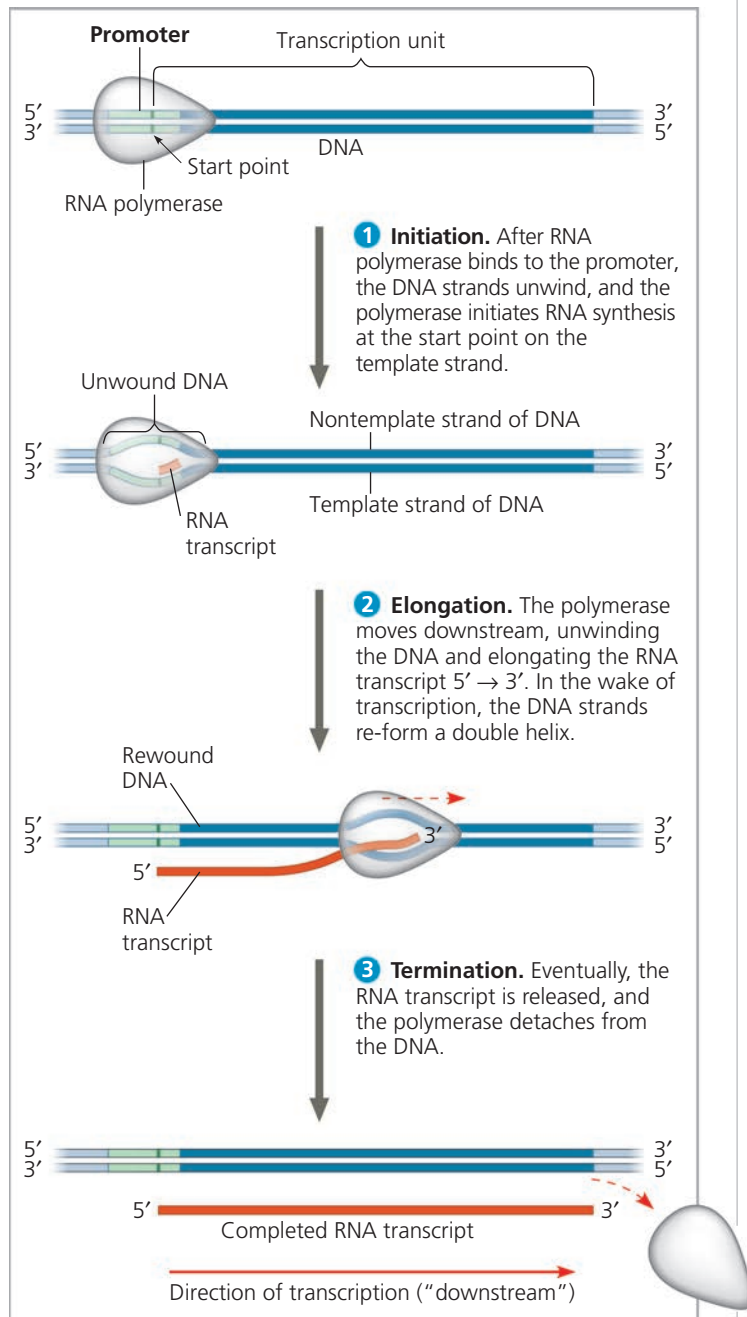
Synthesis of an RNA Transcript

The three stages of transcription, as shown in Figure 17.8 and described next, are initiation, elongation, and termination of the RNA chain. Study Figure 17.8 to familiarize yourself with the stages and the terms used to describe them.

RNA Polymerase Binding and Initiation of Transcription

The promoter of a gene includes within it the transcription **start point**—the nucleotide where RNA polymerase actually begins synthesis of the mRNA—and typically

▼ **Figure 17.8 The stages of transcription: initiation, elongation, and termination.** This general depiction of transcription applies to both bacteria and eukaryotes, but the details of termination differ, as described in the text. Also, in a bacterium, the RNA transcript is immediately usable as mRNA; in a eukaryote, the RNA transcript must first undergo processing.

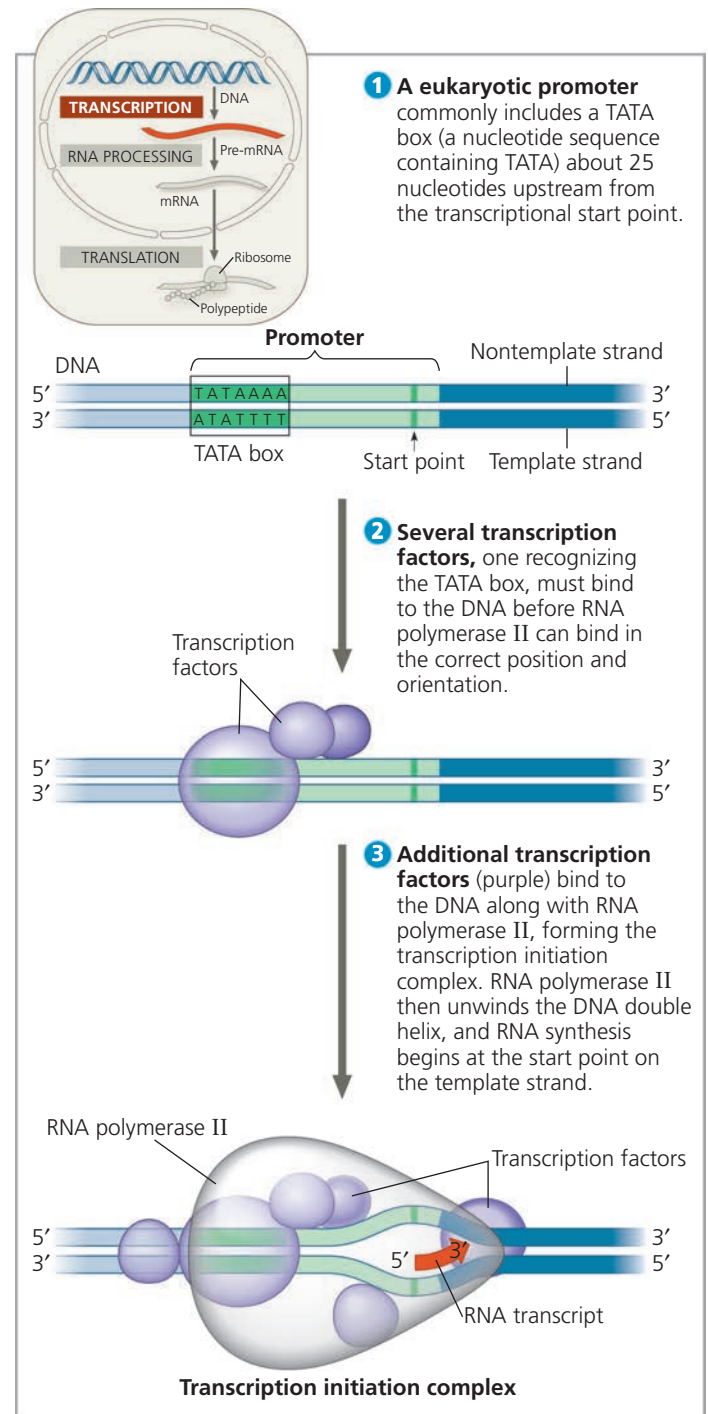


MAKE CONNECTIONS ► Compare the use of a template strand during transcription and replication. See Figure 16.17.

Animation: Overview of Transcription
Animation: Overview of Transcription in Bacteria

extends several dozen or so nucleotide pairs upstream from the start point (**Figure 17.9**). Based on interactions with proteins that will be covered shortly, RNA polymerase binds in a precise location and orientation on the promoter. This in turn determines where transcription starts and which of the two strands of the DNA helix is used as the template.

▼ **Figure 17.9 The initiation of transcription at a eukaryotic promoter.** In eukaryotic cells, proteins called transcription factors mediate the initiation of transcription by RNA polymerase II.



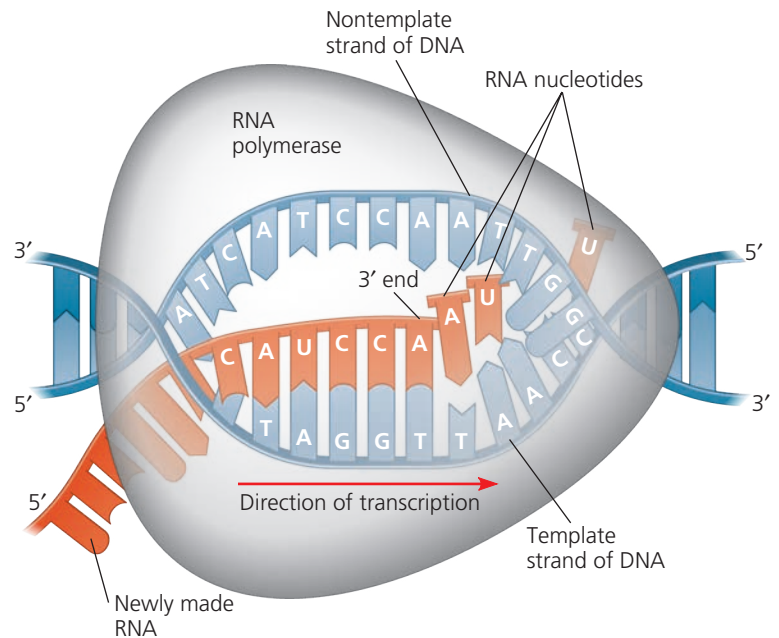
? Explain how the interaction of RNA polymerase with the promoter would differ if the figure showed transcription initiation for bacteria.

The interaction between eukaryotic RNA polymerase II and transcription factors is an example of the importance of protein-protein interactions in controlling eukaryotic transcription. Once the appropriate transcription factors are firmly attached to the promoter DNA and the polymerase is bound to them in the correct orientation on the DNA, the enzyme unwinds the two DNA strands and begins transcribing the template strand at the start point.

As RNA polymerase moves along the DNA, it untwists the double helix, exposing about 10–20 DNA nucleotides at a time for pairing with RNA nucleotides (**Figure 17.10**). The enzyme adds nucleotides to the 3' end of the growing RNA molecule as it continues along the double helix. In the wake of this advancing wave of RNA synthesis, the new RNA molecule peels away from its DNA template, and the DNA double helix re-forms. Transcription progresses at a rate of about 40 nucleotides per second in eukaryotes.

Termination of Transcription

▶ Figure 17.10 Transcription elongation. RNA polymerase moves along the DNA template strand, joining complementary RNA nucleotides to the 3' end of the growing RNA transcript. Behind the polymerase, the new RNA peels away from the template strand, which re-forms a double helix with the nontemplate strand.



before translation. In eukaryotes, RNA polymerase II transcribes a sequence on the DNA called the polyadenylation signal sequence, which specifies a polyadenylation signal (AAUAAA) in the pre-mRNA. This is called a “signal” because once this stretch of six RNA nucleotides appears, it is immediately bound by certain proteins in the nucleus. Then, at a point about 10–35 nucleotides downstream from the AAUAAA, these proteins cut the RNA transcript free from the polymerase, releasing the pre-mRNA. The pre-mRNA then undergoes processing, the topic of the next section. Although that cleavage marks the end of the mRNA, the RNA polymerase II continues to transcribe. Enzymes begin to degrade the RNA starting at its newly exposed 5' end. The polymerase continues transcribing, pursued by the enzymes, until they catch up to the polymerase and it dissociates from the DNA.

1. What is a promoter? Is it located at the upstream or downstream end of a transcription unit?
2. What enables RNA polymerase to start transcribing a gene at the right place on the DNA in a bacterial cell? In a eukaryotic cell?
3. **WHAT IF? ►** Suppose X-rays caused a sequence change in the TATA box of a particular gene's promoter. How would that affect transcription of the gene? (See Figure 17.9.)

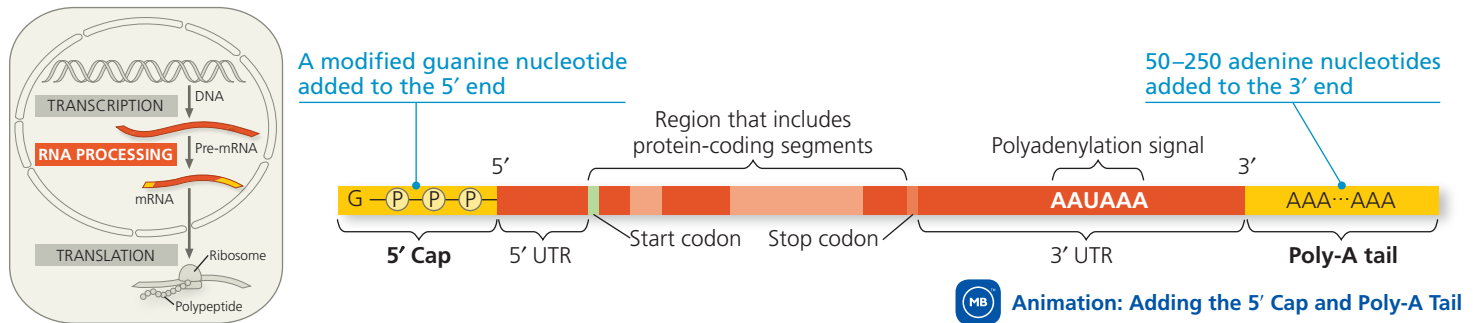
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▼ **Figure 17.11 RNA processing: addition of the 5' cap and poly-A tail.**

Enzymes modify the two ends of a eukaryotic pre-mRNA molecule. The modified ends may promote the export of mRNA from the

nucleus, and they help protect the mRNA from degradation. When the mRNA reaches the cytoplasm, the modified ends, in conjunction with certain cytoplasmic proteins, facilitate ribosome attachment. The 5' cap and poly-A

tail are not translated into protein, nor are the regions called the 5' untranslated region (5' UTR) and 3' untranslated region (3' UTR). The pink segments are introns, which will be described shortly (see Figure 17.12).



CONCEPT 17.3

Eukaryotic cells modify RNA after transcription

Enzymes in the eukaryotic nucleus modify pre-mRNA in specific ways before the genetic message is dispatched to the cytoplasm. During this **RNA processing**, both ends of the primary transcript are altered. Also, in most cases, certain interior sections of the RNA molecule are cut out and the remaining parts spliced together. These modifications produce an mRNA molecule ready for translation.

Alteration of mRNA Ends

Each end of a pre-mRNA molecule is modified in a particular way (**Figure 17.11**). The 5' end, which is synthesized first, receives a **5' cap**, a modified form of a guanine (G) nucleotide added onto the 5' end after transcription of the first 20–40 nucleotides. The 3' end of the pre-mRNA molecule is also

modified before the mRNA exits the nucleus. Recall that the pre-mRNA is cut and released soon after the polyadenylation signal, AAUAAA, is transcribed. At the 3' end, an enzyme then adds 50–250 more adenine (A) nucleotides, forming a **poly-A tail**. The 5' cap and poly-A tail share several important functions. First, they seem to facilitate the export of the mature mRNA from the nucleus. Second, they help protect the mRNA from degradation by hydrolytic enzymes. And third, they help ribosomes attach to the 5' end of the mRNA once the mRNA reaches the cytoplasm. Figure 17.11 also shows the untranslated regions (UTRs) at the 5' and 3' ends of the mRNA (referred to as the 5' UTR and 3' UTR). The UTRs are parts of the mRNA that will not be translated into protein, but they have other functions, such as ribosome binding.

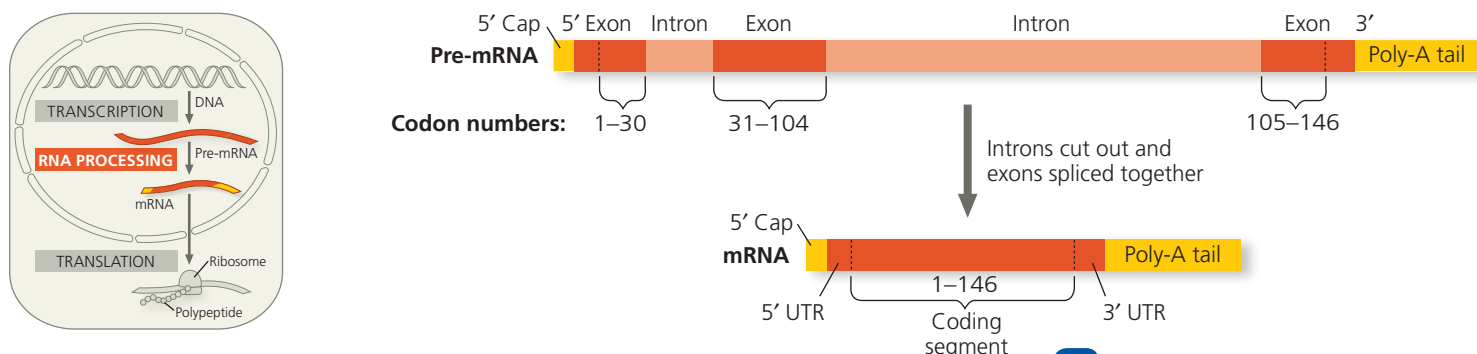
Split Genes and RNA Splicing

A remarkable stage of RNA processing in the eukaryotic nucleus is **RNA splicing** (**Figure 17.12**), where large portions of the RNA molecules are removed and the remaining portions

▼ **Figure 17.12 RNA processing: RNA splicing.** The RNA molecule shown here codes for β -globin, one of the polypeptides of hemoglobin. The numbers under the RNA refer to codons; β -globin is 146 amino acids

long. The β -globin gene and its pre-mRNA transcript have three exons, corresponding to sequences that will leave the nucleus as mRNA. (The 5' UTR and 3' UTR are parts of exons because they are included in the mRNA;

however, they do not code for protein.) During RNA processing, the introns are cut out and the exons spliced together. In many genes, the introns are much longer than the exons.



DRAW IT ► On the mRNA, indicate the sites of the start and stop codons.

BioFlix® Animation: RNA Processing

are reconnected. This cut-and-paste job is similar to editing a movie. The average length of a transcription unit along a human DNA molecule is about 27,000 nucleotide pairs, so the primary RNA transcript is also that long. However, the average-sized protein of 400 amino acids requires only 1,200 nucleotides in RNA to code for it. (Remember, each amino acid is encoded by a *triplet* of nucleotides.) This is because most eukaryotic genes and their RNA transcripts have long noncoding stretches of nucleotides, regions that are not translated. Even more surprising is that most of these noncoding sequences are interspersed between coding segments of the gene and thus between coding segments of the pre-mRNA. In other words, the sequence of DNA nucleotides that codes for a eukaryotic polypeptide is usually not continuous; it is split into segments. The noncoding segments of nucleic acid that lie between coding regions are called *intervening sequences*, or **introns**. The other regions are called **exons**, because they are eventually expressed, usually by being translated into amino acid sequences. (Exceptions include the UTRs of the exons at the ends of the RNA, which make up part of the mRNA but are not translated into protein. Because of these exceptions, you may prefer to think of exons as sequences of RNA that exit the nucleus.) The terms *intron* and *exon* are used for both RNA sequences and the DNA sequences that specify them.

In making a primary transcript from a gene, RNA polymerase II transcribes both introns and exons from the DNA, but the mRNA molecule that enters the cytoplasm is an abridged version. In RNA splicing, the introns are cut out from the molecule and the exons joined together, forming an mRNA molecule with a continuous coding sequence.

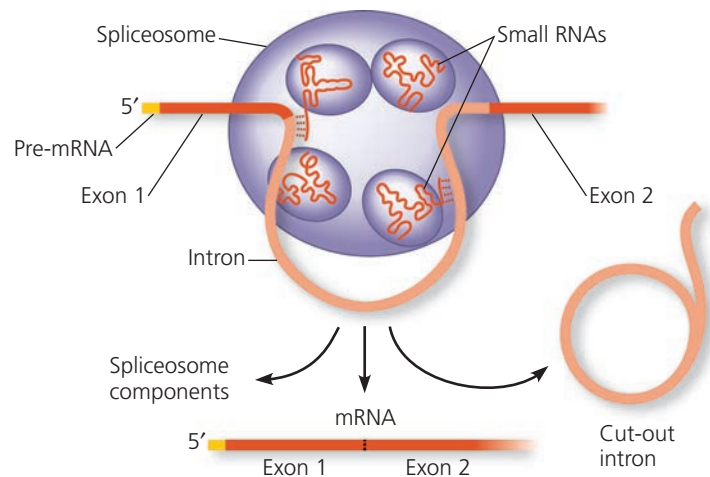
How is pre-mRNA splicing carried out? The removal of introns is accomplished by a large complex made of proteins and small RNAs called a **spliceosome**. This complex binds to several short nucleotide sequences along an intron, including key sequences at each end (Figure 17.13). The intron is then released (and rapidly degraded), and the spliceosome joins together the two exons that flanked the intron. It turns out that the small RNAs in the spliceosome not only participate in spliceosome assembly and splice site recognition, but also catalyze the splicing reaction.

Interview with Joan Steitz: Studying RNA, her “favorite molecule”

Ribozymes

The idea of a catalytic role for the RNAs in the spliceosome arose from the discovery of **ribozymes**, RNA molecules that function as enzymes. In some organisms, RNA splicing can occur without proteins or even additional RNA molecules: The intron RNA functions as a ribozyme and catalyzes its own excision! For example, in the ciliate protist *Tetrahymena*, self-splicing occurs in the production of ribosomal RNA (rRNA), a component of the organism’s

▼ **Figure 17.13 A spliceosome splicing a pre-mRNA.** The diagram shows a portion of a pre-mRNA transcript, with an intron (pink) flanked by two exons (red). Small RNAs within the spliceosome base-pair with nucleotides at specific sites along the intron. Next, small spliceosome RNAs catalyze cutting of the pre-mRNA and the splicing together of the exons, releasing the intron for rapid degradation.



Animation: A Spliceosome

ribosomes. The pre-rRNA actually removes its own introns. The discovery of ribozymes rendered obsolete the idea that all biological catalysts are proteins.

Three properties of RNA enable some RNA molecules to function as enzymes. First, because RNA is single-stranded, a region of an RNA molecule may base-pair, in an antiparallel arrangement, with a complementary region elsewhere in the same molecule; this gives the molecule a particular three-dimensional structure. A specific structure is essential to the catalytic function of ribozymes, just as it is for enzymatic proteins. Second, like certain amino acids in an enzymatic protein, some of the bases in RNA contain functional groups that can participate in catalysis. Third, the ability of RNA to hydrogen-bond with other nucleic acid molecules (either RNA or DNA) adds specificity to its catalytic activity. For example, complementary base pairing between the RNA of the spliceosome and the RNA of a primary RNA transcript precisely locates the region where the ribozyme catalyzes splicing. Later in this chapter, you will see how these properties of RNA also allow it to perform important noncatalytic roles in the cell, such as recognition of the three-nucleotide codons on mRNA.

The Functional and Evolutionary Importance of Introns

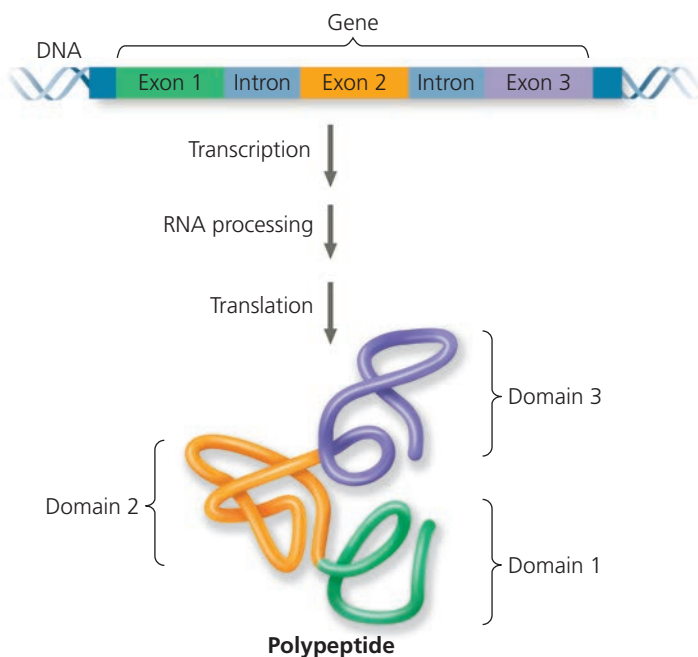
EVOLUTION Whether or not RNA splicing and the presence of introns have provided selective advantages during evolutionary history is a matter of some debate. In any case, it is informative to consider their possible adaptive benefits. Specific functions have not been identified for most introns, but at least some contain sequences that regulate gene expression, and many affect gene products.

One important consequence of the presence of introns in genes is that a single gene can encode more than one kind of polypeptide. Many genes are known to give rise to two or more different polypeptides, depending on which segments are treated as exons during RNA processing; this is called **alternative RNA splicing** (see Figure 18.13). Results from the Human Genome Project (discussed in Concept 20.1) suggest that alternative RNA splicing is one reason humans can get along with about the same number of genes as a nematode (roundworm). Because of alternative splicing, the number of different protein products an organism produces can be much greater than its number of genes.

Proteins often have a modular architecture consisting of discrete structural and functional regions called **domains**. One domain of an enzyme, for example, might include the active site, while another might allow the enzyme to bind to a cellular membrane. In quite a few cases, different exons code for the different domains of a protein (**Figure 17.14**).

The presence of introns in a gene may facilitate the evolution of new and potentially beneficial proteins as a result of a process known as *exon shuffling* (see Figure 20.16). Introns increase the probability of crossing over between the exons of alleles of a gene—simply by providing more terrain for cross-overs without interrupting coding sequences. This might result in new combinations of exons and proteins with altered structure and function. We can also imagine the occasional mixing and matching of exons between completely different (nonallelic) genes. Exon shuffling of either sort could lead to new proteins with novel combinations of functions. While most of the shuffling would result in nonbeneficial changes, occasionally a beneficial variant might arise.

▼ **Figure 17.14** Correspondence between exons and protein domains.



CONCEPT CHECK 17.3

1. There are about 20,000 human protein-coding genes. How can human cells make 75,000–100,000 different proteins?
2. How is RNA splicing similar to how you would watch a recorded television show? What would introns be?
3. **WHAT IF?** > What would be the effect of treating cells with an agent that removed the cap from mRNAs?

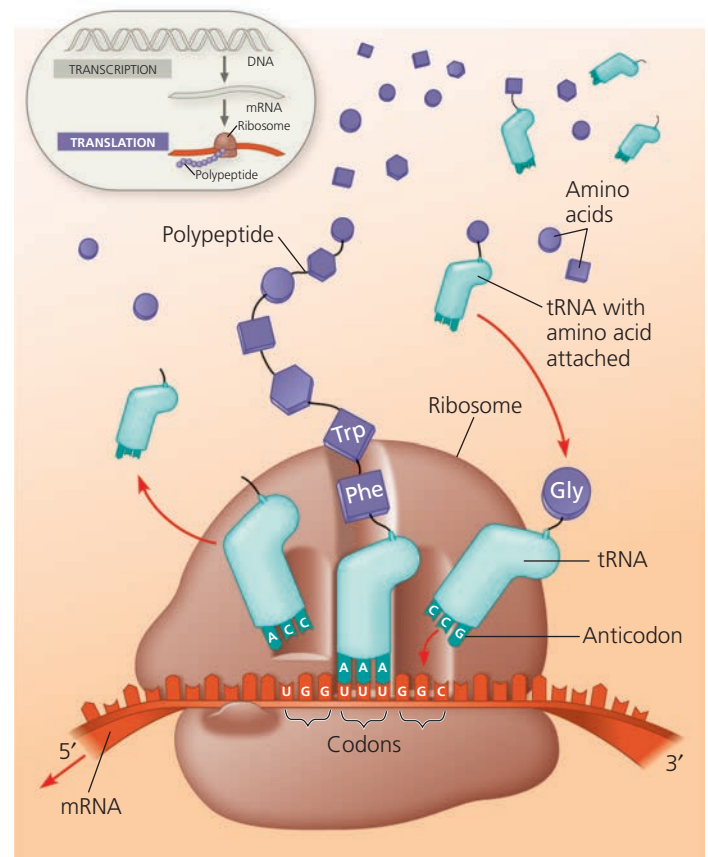
For suggested answers, see Appendix A.

CONCEPT 17.4

Translation is the RNA-directed synthesis of a polypeptide: a closer look

We will now examine how genetic information flows from mRNA to protein—the process of translation (**Figure 17.15**). We'll focus on the basic steps of translation that occur in both bacteria and eukaryotes, while pointing out key differences.

▼ **Figure 17.15** Translation: the basic concept. As a molecule of mRNA is moved through a ribosome, codons are translated into amino acids, one by one. The translators, or interpreters, are tRNA molecules, each type with a specific anticodon at one end and a corresponding amino acid at the other end. A tRNA adds its amino acid cargo to a growing polypeptide chain when the anticodon hydrogen-bonds to the complementary codon on the mRNA.



BioFlix® Animation: Translation
Animation: Overview of Translation

Molecular Components of Translation

In the process of translation, a cell “reads” a genetic message and builds a polypeptide accordingly. The message is a series of codons along an mRNA molecule, and the translator is called a **transfer RNA (tRNA)**. The function of a tRNA is to transfer an amino acid from the cytoplasmic pool of amino acids to a growing polypeptide in a ribosome. A cell keeps its cytoplasm stocked with all 20 amino acids, either by synthesizing them from other compounds or by taking them up from the surrounding solution. The ribosome, a structure made of proteins and RNAs, adds each amino acid brought to it by a tRNA to the growing end of a polypeptide chain (see Figure 17.15).

Translation is simple in principle but complex in its biochemistry and mechanics, especially in the eukaryotic cell. In dissecting translation, we’ll focus on the slightly less complicated version of the process that occurs in bacteria. We’ll first look at the major players in this process.

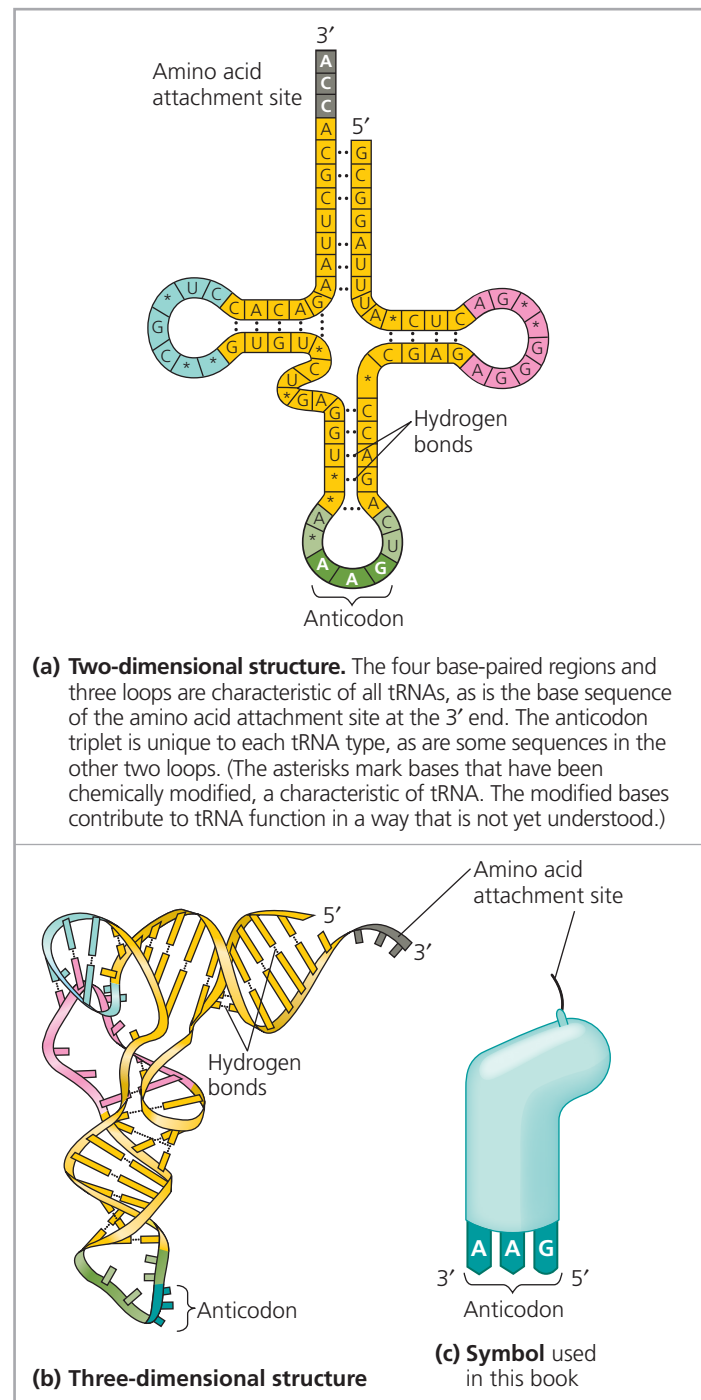
The Structure and Function of Transfer RNA

The key to translating a genetic message into a specific amino acid sequence is the fact that each tRNA molecule enables translation of a given mRNA codon into a certain amino acid. This is possible because a tRNA bears a specific amino acid at one end of its three-dimensional structure, while at the other end is a nucleotide triplet that can base-pair with the complementary codon on mRNA.

A tRNA molecule consists of a single RNA strand that is only about 80 nucleotides long (compared to hundreds of nucleotides for most mRNA molecules). Because of the presence of complementary stretches of nucleotide bases that can hydrogen-bond to each other, this single strand can fold back on itself and form a molecule with a three-dimensional structure. Flattened into one plane to clarify this base pairing, a tRNA molecule looks like a cloverleaf (Figure 17.16a). The tRNA actually twists and folds into a compact three-dimensional structure that is roughly L-shaped (Figure 17.16b), with the 5′ and 3′ ends of the linear tRNA both located near one end of the structure. The protruding 3′ end acts as the attachment site for an amino acid. The loop extending from the other end of the L includes the **anticodon**, the particular nucleotide triplet that base-pairs to a specific mRNA codon. Thus, the structure of a tRNA molecule fits its function.

Anticodons are conventionally written 3′ → 5′ to align properly with codons written 5′ → 3′ (see Figure 17.15). (For base pairing, RNA strands must be antiparallel, like DNA.) As an example of how tRNAs work, consider the mRNA codon 5′-GGC-3′, which is translated as the amino acid glycine. The tRNA that base-pairs with this codon by hydrogen bonding has 3′-CCG-5′ as its anticodon and carries glycine at its other end (see the incoming tRNA approaching the ribosome in

▼ **Figure 17.16** The structure of transfer RNA (tRNA).



VISUAL SKILLS ► Look at the tRNA shown in this figure. Based on its anticodon, identify the codon it would bind to, as well as the amino acid that it would carry.



Figure 17.15). As an mRNA molecule is moved through a ribosome, glycine will be added to the polypeptide chain whenever the codon 5′-GGC-3′ is presented for translation. Codon by codon, the genetic message is translated as tRNAs

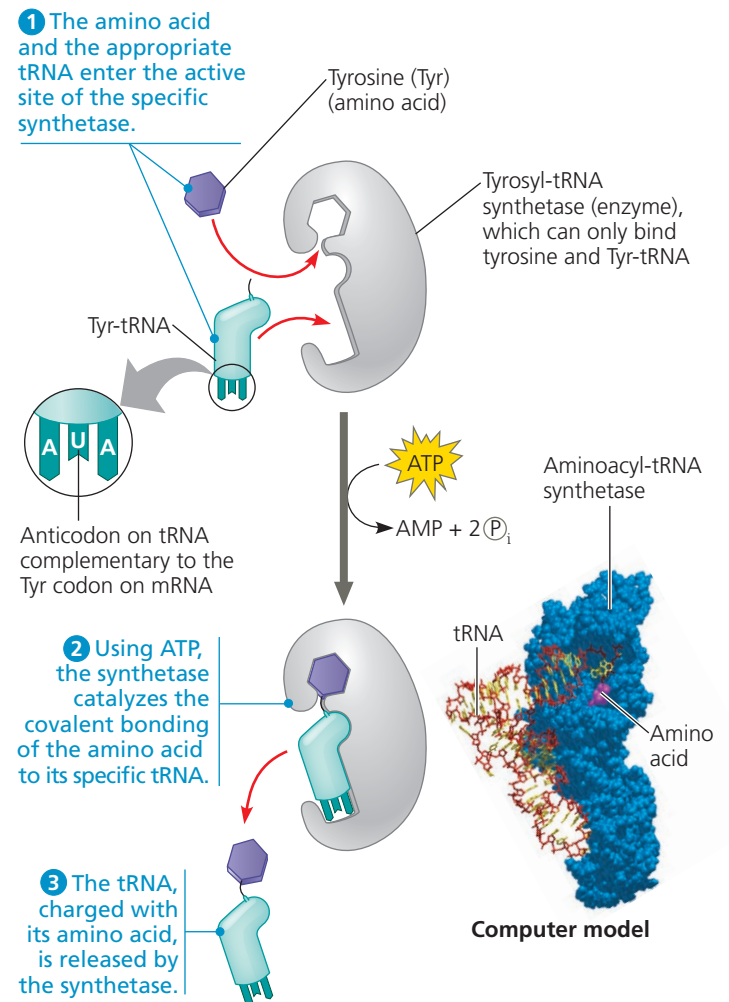
position each amino acid in the order prescribed, and the ribosome adds that amino acid onto the growing polypeptide chain. The tRNA molecule is a translator in the sense that, in the context of the ribosome, it can read a nucleic acid word (the mRNA codon) and interpret it as a protein word (the amino acid).

Like mRNA and other types of cellular RNA, transfer RNA molecules are transcribed from DNA templates. In a eukaryotic cell, tRNA, like mRNA, is made in the nucleus and then travels to the cytoplasm, where it will participate in the process of translation. In both bacterial and eukaryotic cells, each tRNA molecule is used repeatedly, picking up its designated amino acid in the cytosol, depositing this cargo onto a polypeptide chain at the ribosome, and then leaving the ribosome, ready to pick up another of the same amino acid.

The accurate translation of a genetic message requires two instances of molecular recognition. First, a tRNA that binds to an mRNA codon specifying a particular amino acid must carry that amino acid, and no other, to the ribosome. The correct matching up of tRNA and amino acid is carried out by a family of related enzymes that are aptly named **aminoacyl-tRNA synthetases** (Figure 17.17). The active site of each type of aminoacyl-tRNA synthetase fits only a specific combination of amino acid and tRNA. There are 20 different synthetases, one for each amino acid. A synthetase joins a given amino acid to an appropriate tRNA; one synthetase is able to bind to all the different tRNAs for its particular amino acid. The synthetase catalyzes the covalent attachment of the amino acid to its tRNA in a process driven by the hydrolysis of ATP. The resulting aminoacyl tRNA, also called a charged tRNA, is released from the enzyme and is then available to deliver its amino acid to a growing polypeptide chain on a ribosome.

The second instance of molecular recognition is the pairing of the tRNA anticodon with the appropriate mRNA codon. If one tRNA variety existed for each mRNA codon specifying an amino acid, there would be 61 tRNAs (see Figure 17.6). In bacteria, however, there are only about 45 tRNAs, signifying that some tRNAs must be able to bind to more than one codon. Such versatility is possible because the rules for base pairing between the third nucleotide base of a codon and the corresponding base of a tRNA anticodon are relaxed compared to those at other codon positions. For example, the nucleotide base U at the 5' end of a tRNA anticodon can pair with either A or G in the third position (at the 3' end) of an mRNA codon. The flexible base pairing at this codon position is called **wobble**. Wobble explains why the synonymous codons for a given amino acid most often differ in their third nucleotide base. Accordingly, a tRNA with the anticodon 3'-UCU-5' can base-pair with either the mRNA codon 5'-AGA-3' or 5'-AGG-3', both of which code for arginine (see Figure 17.6).

▼ **Figure 17.17 Aminoacyl-tRNA synthetases provide specificity in joining amino acids to their tRNAs.** Linkage of a tRNA to its amino acid is an endergonic process that occurs at the expense of ATP, which loses two phosphate groups, becoming AMP.



Animation: Transfer RNA

The Structure and Function of Ribosomes

Ribosomes facilitate the specific coupling of tRNA anticodons with mRNA codons during protein synthesis. A ribosome consists of a large subunit and a small subunit, each made up of proteins and one or more **ribosomal RNAs (rRNAs)**. In eukaryotes, the subunits are made in the nucleolus. Ribosomal RNA genes are transcribed, and the RNA is processed and assembled with proteins imported from the cytoplasm. Completed ribosomal subunits are then exported via nuclear pores to the cytoplasm. In both bacteria and eukaryotes, a large and a small subunit join to form a functional ribosome only when attached to an mRNA molecule. About one-third of the mass of a ribosome is made up of proteins; the rest consists of three rRNA molecules (in bacteria) or four (in eukaryotes). Because most cells contain thousands of ribosomes, rRNA is the most abundant type of cellular RNA.

Although the ribosomes of bacteria and eukaryotes are very similar in structure and function, eukaryotic ribosomes are slightly larger, as well as differing somewhat from bacterial ribosomes in their molecular composition. The differences are medically significant. Certain antibiotic drugs can inactivate bacterial ribosomes without affecting eukaryotic ribosomes. These drugs, including tetracycline and streptomycin, are used to combat bacterial infections.

The structure of a ribosome reflects its function of bringing mRNA together with tRNAs carrying amino acids. In addition to a binding site for mRNA, each ribosome has three binding sites for tRNA (**Figure 17.18**). The **P site** (peptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain, while the **A site** (aminoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the **E site** (exit site). The ribosome holds the tRNA and mRNA in close proximity and positions the new amino acid so that it can be added to the carboxyl end of the growing polypeptide. It then catalyzes the formation of the peptide bond. As the polypeptide becomes longer, it passes through an *exit tunnel* in the ribosome's large subunit. When the polypeptide is complete, it is released through the exit tunnel.

The widely accepted model is that rRNAs, rather than ribosomal proteins, are primarily responsible for both the structure and the function of the ribosome. The proteins, which are largely on the exterior, support the shape changes of the rRNA molecules as they carry out catalysis during translation. Ribosomal RNA is the main constituent of the A and P sites and of the interface between the two subunits; it also acts as the catalyst of peptide bond formation. Thus, a ribosome could actually be considered one colossal ribozyme!

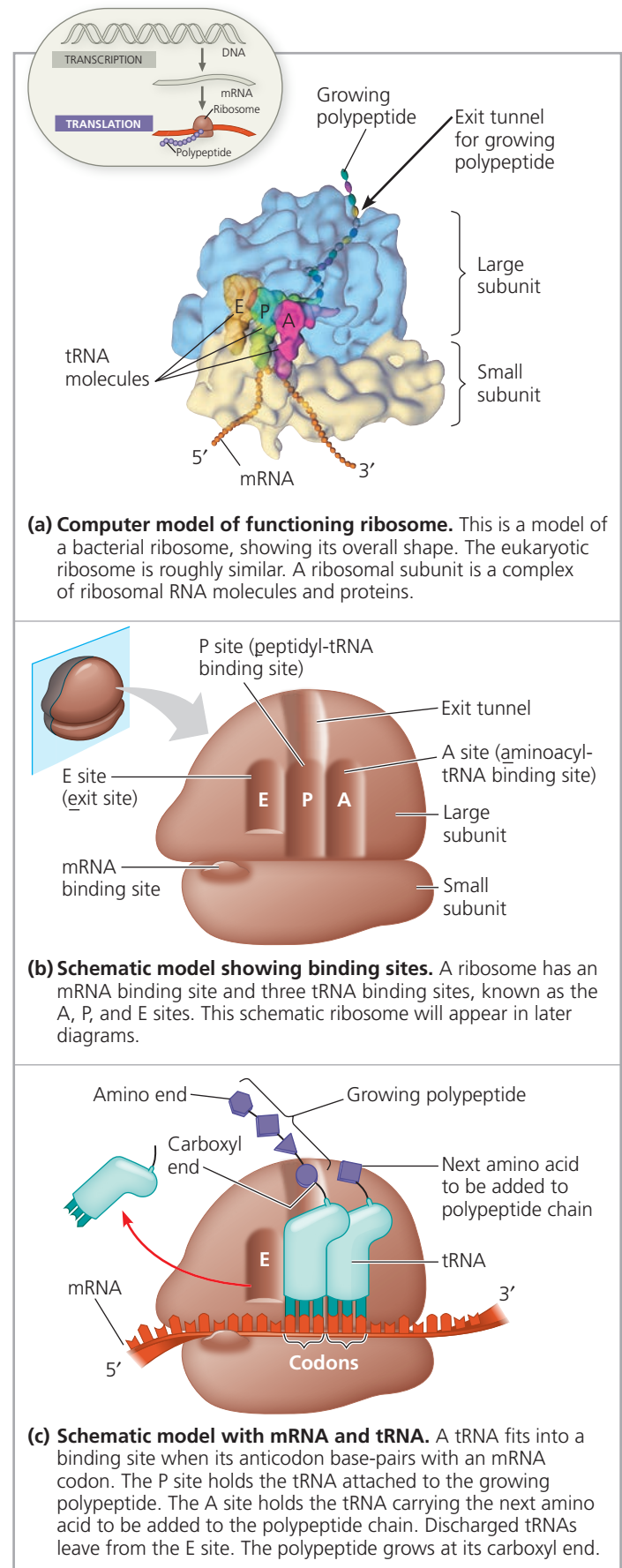
Building a Polypeptide

We can divide translation, the synthesis of a polypeptide, into three stages: initiation, elongation, and termination. All three require protein “factors” that aid in the translation process. Some steps of initiation and elongation also require energy, provided by the hydrolysis of guanosine triphosphate (GTP).

Ribosome Association and Initiation of Translation

In either bacteria or eukaryotes, the start codon (AUG) signals the start of translation; this is important because it establishes the codon reading frame for the mRNA. In the first step of translation, a small ribosomal subunit binds to both the mRNA and a specific initiator tRNA, which carries the amino acid methionine. In bacteria, the small subunit can bind the two in either order; it binds the mRNA at a specific RNA sequence, just upstream of the AUG start codon. In the **Scientific Skills Exercise**, you can work with DNA sequences encoding the ribosomal binding sites on the mRNAs of a group of *Eschericia coli*

▼ **Figure 17.18** The anatomy of a functioning ribosome.



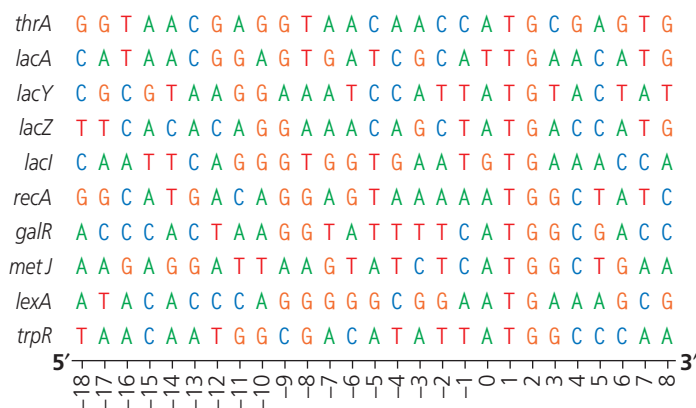
SCIENTIFIC SKILLS EXERCISE

Interpreting a Sequence Logo

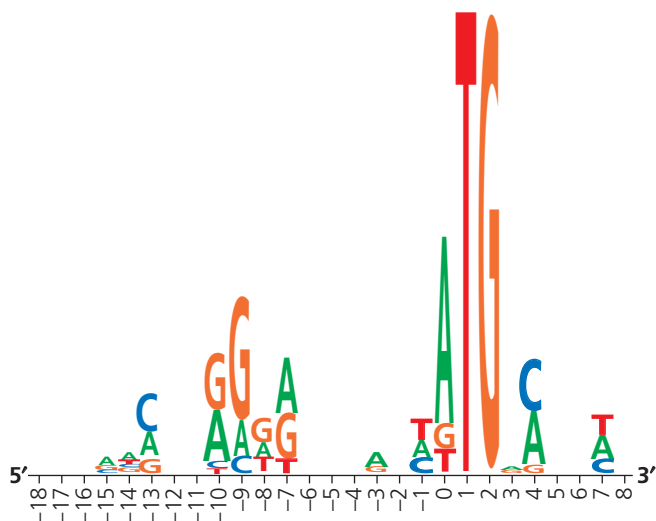
How Can a Sequence Logo Be Used to Identify Ribosome Binding Sites on Bacterial mRNAs? When initiating translation, ribosomes bind to an mRNA at a ribosome binding site upstream of the AUG start codon. Because mRNAs from different genes all bind to a ribosome, the genes encoding these mRNAs are likely to have a similar base sequence where the ribosomes bind. Therefore, candidate ribosome binding sites on mRNA can be identified by comparing DNA sequences (and thus the mRNA sequences) of multiple genes in a species, searching the region upstream of the start codon for shared (conserved) stretches of bases. In this exercise, you will analyze DNA sequences from multiple such genes, represented by a visual graphic called a sequence logo.

How the Experiment Was Done The DNA sequences of 149 genes from the *E. coli* genome were aligned using computer software. The aim was to identify similar base sequences—at the appropriate location in each gene—as potential ribosome binding sites. Rather than presenting the data as a series of 149 sequences aligned in a column (a sequence alignment), the researchers used a sequence logo.

Data from the Experiment To show how sequence logos are made, the potential ribosome binding regions from 10 *E. coli* genes are shown below in a sequence alignment, followed by the sequence logo derived from the aligned sequences. Note that the DNA shown is the nontemplate (coding) strand, which is how DNA sequences are typically presented.



▲ Sequence alignment



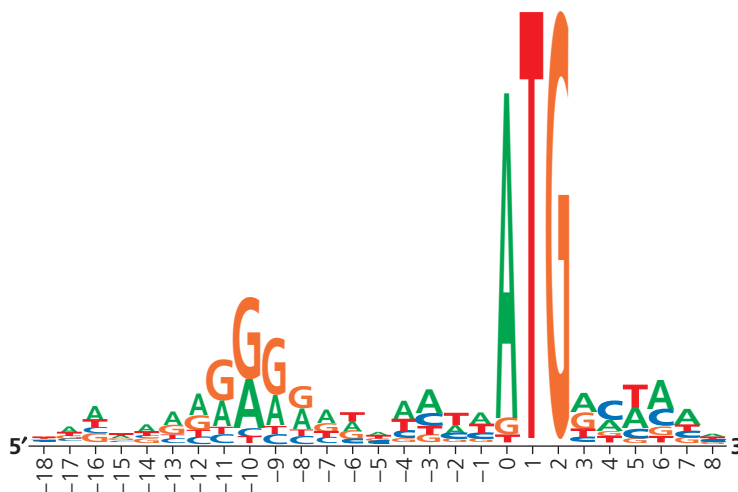
▲ Sequence logo

INTERPRET THE DATA

Ribosome binding site on mRNA



- In the sequence logo (bottom, left), the horizontal axis shows the primary sequence of the DNA by nucleotide position. Letters for each base are stacked on top of each other according to their relative frequency at that position among the aligned sequences, with the most common base as the largest letter at the top of the stack. The height of each letter represents the relative frequency of that base at that position. (a) In the sequence alignment, count the number of each base at position -9 and order them from most to least frequent. Compare this to the size and placement of each base at -9 in the logo. (b) Do the same for positions 0 and 1.
- The height of a stack of letters in a logo indicates the predictive power of that stack (determined statistically). If the stack is tall, we can be more confident in predicting what base will be in that position if a new sequence is added to the logo. For example, at position 2 in the sequence alignment, all 10 sequences have a G; the probability of finding a G there in a new sequence is very high, as is the stack in the sequence logo. For short stacks, the bases all have about the same frequency, so it's hard to predict a base at those positions. (a) Looking at the sequence logo, which two positions have the most predictable bases? What bases do you predict would be at those positions in a newly sequenced gene? (b) Which 12 positions have the least predictable bases? How do you know? How does this reflect the relative frequencies of the bases shown at these positions in the sequence alignment? Use the two leftmost positions of the 12 as examples in your answer.
- In the actual experiment, the researchers used 149 sequences to build their sequence logo, which is shown below. There is a stack at each position, even if short, because the sequence logo includes more data. (a) Which three positions in this sequence logo have the most predictable bases? Name the most frequent base at each. (b) Which four positions have the least predictable bases? How can you tell?



- A consensus sequence identifies the base occurring most often at each position in the set of sequences. (a) Write out the consensus sequence of this (the nontemplate) strand. In any position where the base can't be determined, put a dash. (b) Which provides more information—the consensus sequence or the sequence logo? What is lost in the less informative method?
- (a) Based on the logo, what five adjacent base positions in the 5' UTR region are most likely to be involved in ribosome binding? Explain. (b) What is represented by the bases in positions 0–2?

Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Further Reading T. D. Schneider and R. M. Stephens, Sequence logos: A new way to display consensus sequences, *Nucleic Acids Research* 18:6097–6100 (1990).

genes. In eukaryotes, the small subunit, with the initiator tRNA already bound, binds to the 5' cap of the mRNA and then moves, or *scans*, downstream along the mRNA until it reaches the start codon; the initiator tRNA then hydrogen-bonds to the AUG start codon.

Thus, the first components to associate with each other during the initiation stage of translation are mRNA, a tRNA bearing the first amino acid of the polypeptide, and the small ribosomal subunit (**Figure 17.19**). This is followed by the attachment of a large ribosomal subunit, completing the *translation initiation complex*. Proteins called *initiation factors* are required to bring all these components together. The cell also expends energy obtained by hydrolysis of a GTP molecule to form the initiation complex. At the completion of the initiation process, the initiator tRNA sits in the P site of the ribosome, and the vacant A site is ready for the next aminoacyl tRNA. Note that a polypeptide is always synthesized in one direction, from the initial methionine at the amino end, also called the N-terminus, toward the final amino acid at the carboxyl end, also called the C-terminus (see Figure 5.15).

Elongation of the Polypeptide Chain

In the elongation stage of translation, amino acids are added one by one to the previous amino acid at the C-terminus of the growing chain. Each addition involves several proteins called *elongation factors* and occurs in a three-step cycle described in **Figure 17.20**. Energy expenditure occurs in the first and third steps. Codon recognition requires hydrolysis of one molecule of GTP, which increases the accuracy and efficiency of this step. One more GTP is hydrolyzed to provide energy for the translocation step.

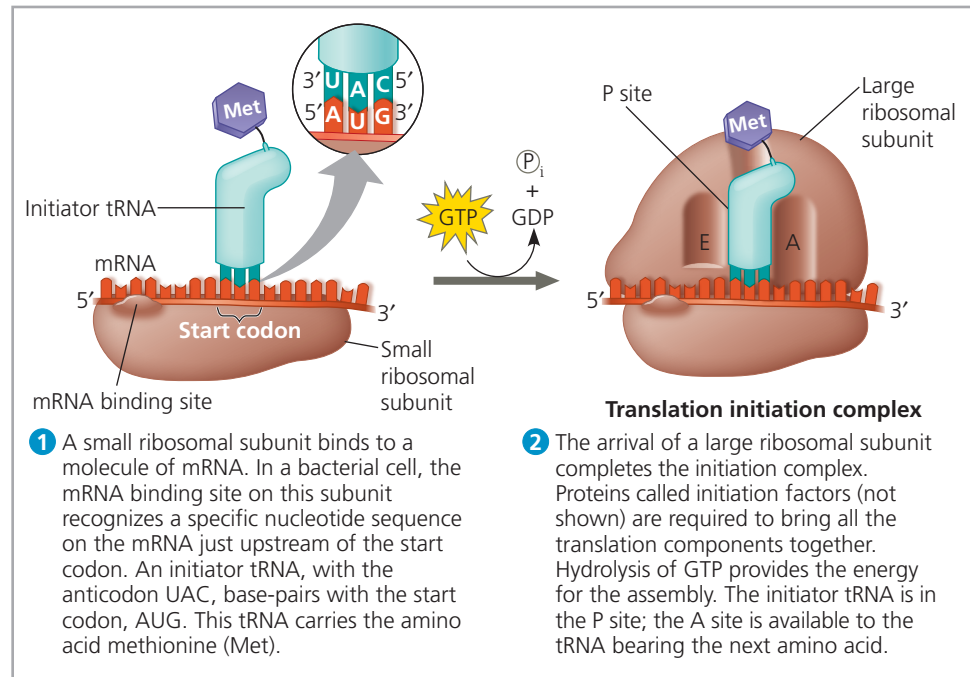
The mRNA is moved through the ribosome in one direction only, 5' end first; this is equivalent to the ribosome moving 5' → 3' on the mRNA. The main point is that the ribosome and the mRNA move relative to each other, unidirectionally, codon by codon. The elongation cycle takes less than a tenth of a second in bacteria and is repeated as each amino acid is added until the polypeptide is complete. The empty tRNAs that are released from the E site return to the cytoplasm, where they will be reloaded with the appropriate amino acid (see Figure 17.17).

Termination of Translation

The final stage of translation is termination (**Figure 17.21**). Elongation continues until a stop codon in the mRNA reaches the A site. The nucleotide base triplets UAG, UAA,

▼ **Figure 17.19** The initiation of translation.

 **Animation: Initiation of Translation**



and UGA (all written 5' → 3') do not code for amino acids but instead act as signals to stop translation. A *release factor*, a protein shaped like an aminoacyl tRNA, binds directly to the stop codon in the A site. The release factor causes the addition of a water molecule instead of an amino acid to the polypeptide chain. (Water molecules are abundant in the cytosol.) This reaction breaks (hydrolyzes) the bond between the completed polypeptide and the tRNA in the P site, releasing the polypeptide through the exit tunnel of the ribosome's large subunit. The remainder of the translation assembly then comes apart in a multistep process, aided by other protein factors. Breakdown of the translation assembly requires the hydrolysis of two more GTP molecules.

Completing and Targeting the Functional Protein

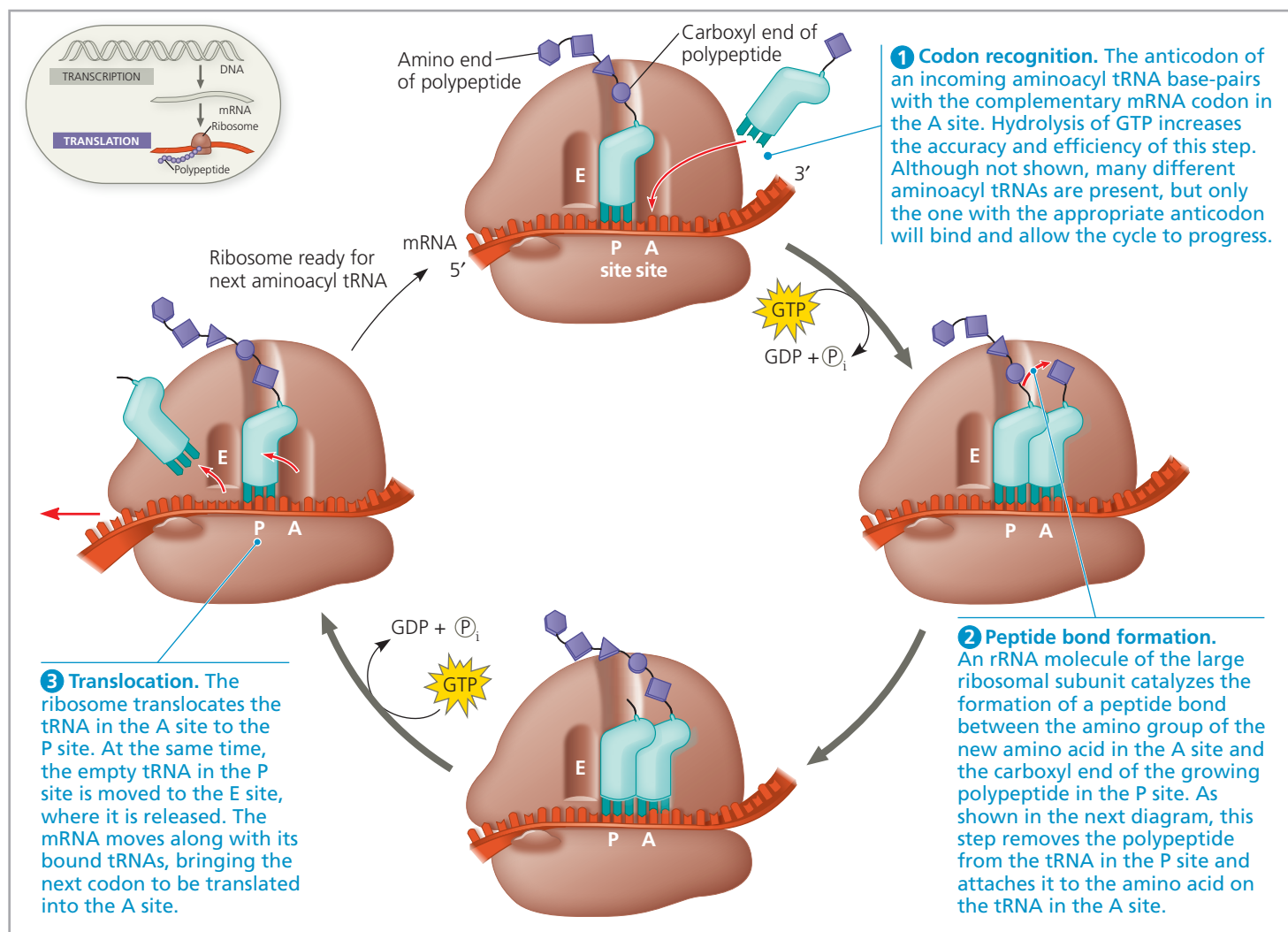
The process of translation is often not sufficient to make a functional protein. In this section, you will learn about modifications that polypeptide chains undergo after the translation process as well as some of the mechanisms used to target completed proteins to specific sites in the cell.

Protein Folding and Post-Translational Modifications

During its synthesis, a polypeptide chain begins to coil and fold spontaneously as a consequence of its amino acid sequence (primary structure), forming a protein with a specific shape: a three-dimensional molecule with secondary and tertiary structure (see Figure 5.18). Thus, a gene determines primary structure, which in turn determines shape.

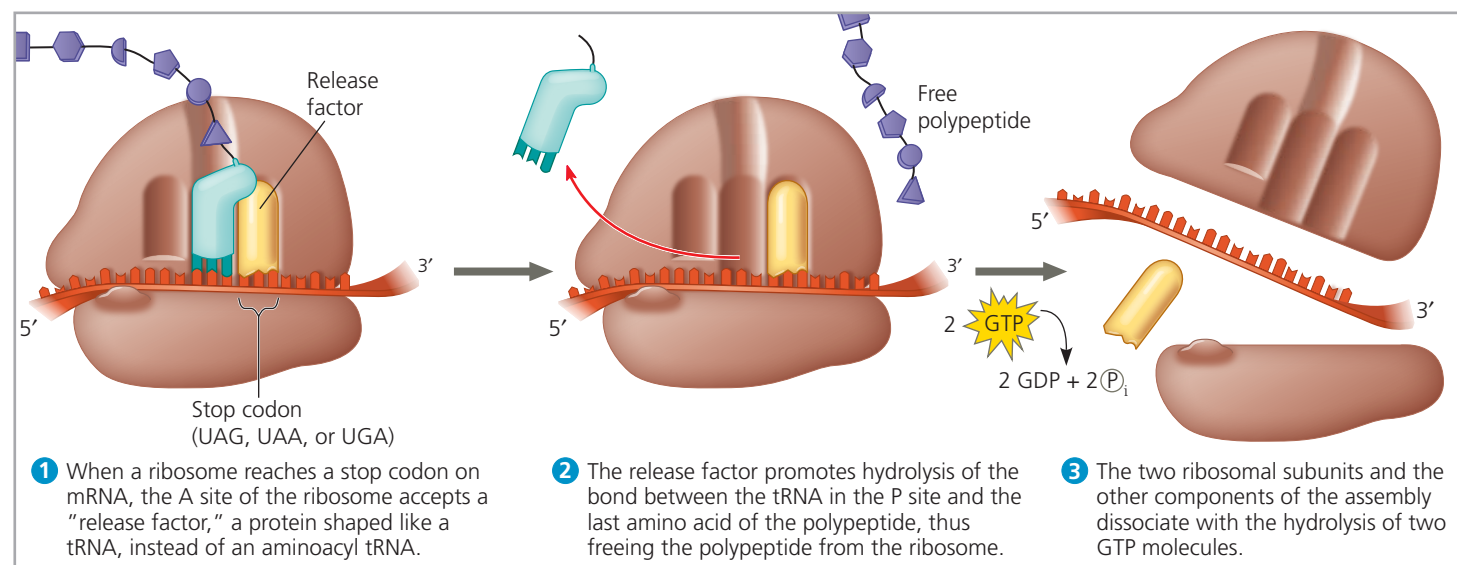
▼ **Figure 17.20 The elongation cycle of translation.** The hydrolysis of GTP plays an important role in the elongation process; elongation factors are not shown.

 **Animation: Elongation Cycle of Translation**



▼ **Figure 17.21 The termination of translation.** Like elongation, termination requires GTP hydrolysis as well as additional protein factors, which are not shown here.

 **Animation: Termination of Translation**



Additional steps—*post-translational modifications*—may be required before the protein can begin doing its particular job in the cell. Certain amino acids may be chemically modified by the attachment of sugars, lipids, phosphate groups, or other additions. Enzymes may remove one or more amino acids from the leading (amino) end of the polypeptide chain. In some cases, a polypeptide chain may be enzymatically cleaved into two or more pieces. In other cases, two or more polypeptides that are synthesized separately may come together, if the protein has quaternary structure; an example is hemoglobin (see Figure 5.18).



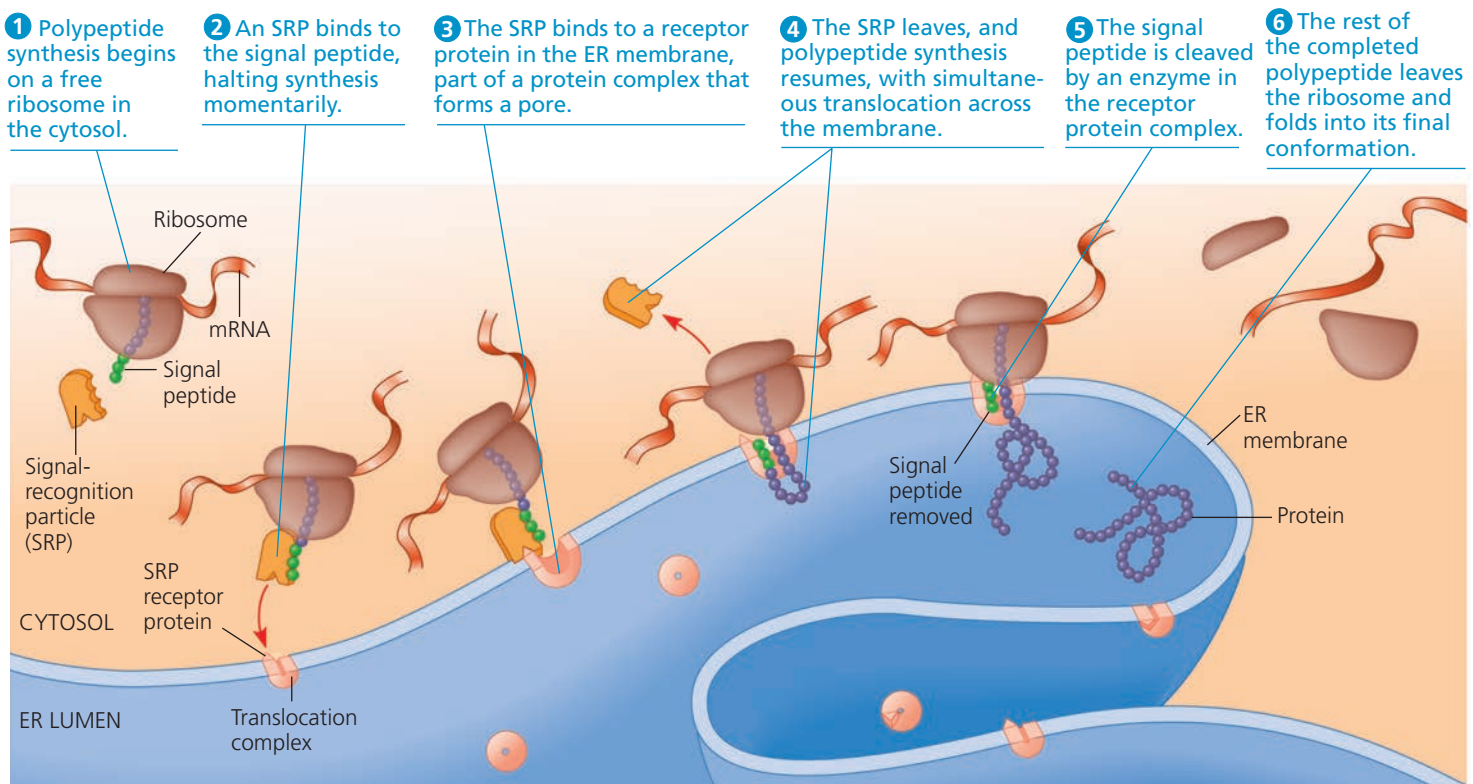
Targeting Polypeptides to Specific Locations

In electron micrographs of eukaryotic cells active in protein synthesis, two populations of ribosomes are evident: free and bound (see Figure 7.10). Free ribosomes are suspended in the cytosol and mostly synthesize proteins that stay in the cytosol and function there. In contrast, bound ribosomes are attached to the cytosolic side of the endoplasmic reticulum (ER) or to the nuclear envelope. Bound ribosomes make proteins of the endomembrane system (see Figure 7.15) as well as proteins secreted from the cell, such as insulin. It is important to note that the ribosomes themselves are identical and can alternate between being free ribosomes one time they are used and being bound ribosomes the next.

What determines whether a ribosome is free in the cytosol or bound to rough ER? Polypeptide synthesis always begins in the cytosol as a free ribosome starts to translate an mRNA molecule. There, the process continues to completion—*unless* the growing polypeptide itself cues the ribosome to attach to the ER. The polypeptides of proteins destined for the endomembrane system or for secretion are marked by a **signal peptide**, which targets the protein to the ER (**Figure 17.22**). The signal peptide, a sequence of about 20 amino acids at or near the leading end (N-terminus) of the polypeptide, is recognized as it emerges from the ribosome by a protein-RNA complex called a **signal-recognition particle (SRP)**. This particle escorts the ribosome to a receptor protein built into the ER membrane. The receptor is part of a multiprotein translocation complex. Polypeptide synthesis continues there, and the growing polypeptide snakes across the membrane into the ER lumen via a protein pore. The rest of the completed polypeptide, if it is to be secreted from the cell, is released into solution within the ER lumen. Alternatively, if the polypeptide is to be a membrane protein, it remains partially embedded in the ER membrane. In either case, it travels in a transport vesicle to its destination (see, for example, Figure 8.9).

Other kinds of signal peptides are used to target polypeptides to mitochondria, chloroplasts, the interior of the nucleus, and other organelles that are not part of the endomembrane system. The critical difference in these cases is that translation is completed in the cytosol before the polypeptide is imported

▼ **Figure 17.22** The signal mechanism for targeting proteins to the ER.



MAKE CONNECTIONS ► If this protein were destined for secretion, what would happen to it after its synthesis was completed? See Figure 8.9.



into the organelle. Translocation mechanisms also vary, but in all cases studied to date, the “postal zip codes” that address proteins for secretion or to cellular locations are signal peptides of some sort. Bacteria also employ signal peptides to target proteins to the plasma membrane for secretion.

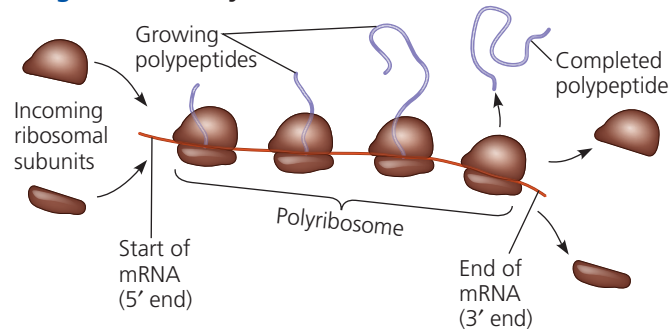
Making Multiple Polypeptides in Bacteria and Eukaryotes

In previous sections, you learned how a single polypeptide is synthesized using the information encoded in an mRNA molecule. When a polypeptide is required in a cell, though, the need is for many copies, not just one.

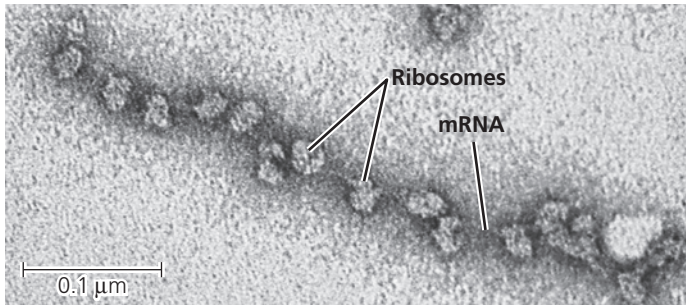
A single ribosome can make an average-sized polypeptide in less than a minute. In both bacteria and eukaryotes, however, multiple ribosomes translate an mRNA at the same time (**Figure 17.23**); that is, a single mRNA is used to make many copies of a polypeptide simultaneously. Once a ribosome is far enough past the start codon, a second ribosome can attach to the mRNA, eventually resulting in a number of ribosomes trailing along the mRNA. Such strings of ribosomes, called **polyribosomes** (or **polysomes**), can be seen with an electron microscope; they can be either free or bound. They enable a cell to rapidly make many copies of a polypeptide.

Another way both bacteria and eukaryotes augment the number of copies of a polypeptide is by transcribing multiple mRNAs from the same gene. However, the coordination of the two processes—transcription and translation—differs in the two groups. The most important differences between bacteria

▼ **Figure 17.23 Polyribosomes.**

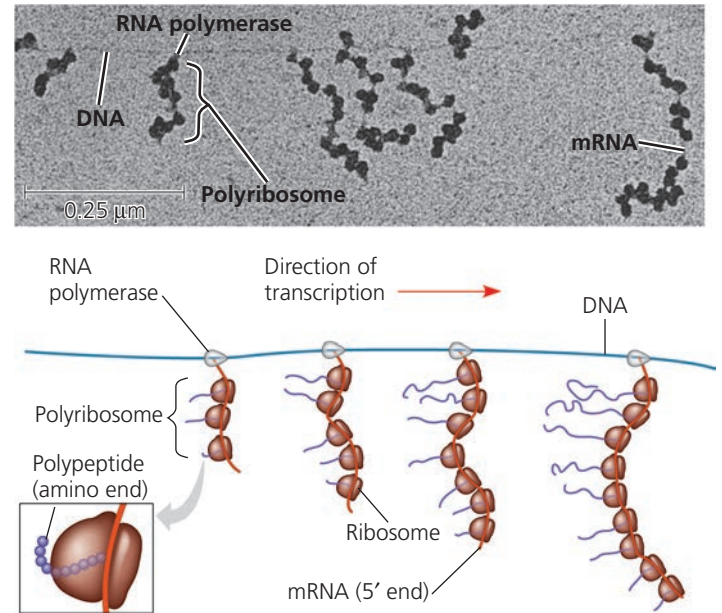


(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a bacterial cell. Growing polypeptides are not visible here (TEM).

▼ **Figure 17.24 Coupled transcription and translation in bacteria.** In bacterial cells, the translation of mRNA can begin as soon as the leading (5') end of the mRNA molecule peels away from the DNA template. The micrograph (TEM) shows a strand of *E. coli* DNA being transcribed by RNA polymerase molecules. Attached to each RNA polymerase molecule is a growing strand of mRNA, which is already being translated by ribosomes. The newly synthesized polypeptides are not visible in the micrograph but are shown in the diagram.



VISUAL SKILLS ► Which one of the mRNA molecules started being transcribed first? On that mRNA, which ribosome started translating the mRNA first?

and eukaryotes arise from the bacterial cell's lack of compartmental organization. Like a one-room workshop, a bacterial cell ensures a streamlined operation by coupling the two processes. In the absence of a nucleus, it can simultaneously transcribe and translate the same gene (**Figure 17.24**), and the newly made protein can quickly diffuse to its site of function.

In contrast, the eukaryotic cell's nuclear envelope segregates transcription from translation and provides a compartment for extensive RNA processing. This processing stage includes additional steps, discussed earlier, the regulation of which can help coordinate the eukaryotic cell's elaborate activities. **Figure 17.25** summarizes the path from gene to polypeptide in a eukaryotic cell.

CONCEPT CHECK 17.4

1. What two processes ensure that the correct amino acid is added to a growing polypeptide chain?
2. Discuss the different post-translational changes that may be needed to make a functional protein.
3. **WHAT IF? DRAW IT** ► Draw a tRNA with the anticodon 3'-CGU-5'. What two different codons could it bind to? Draw each codon on an mRNA, labeling all 5' and 3' ends, the tRNA, and the amino acid it carries.
4. **WHAT IF?** ► In eukaryotic cells, mRNAs have been found to have a circular arrangement in which proteins hold the poly-A tail near the 5' cap. How might this increase translation efficiency?

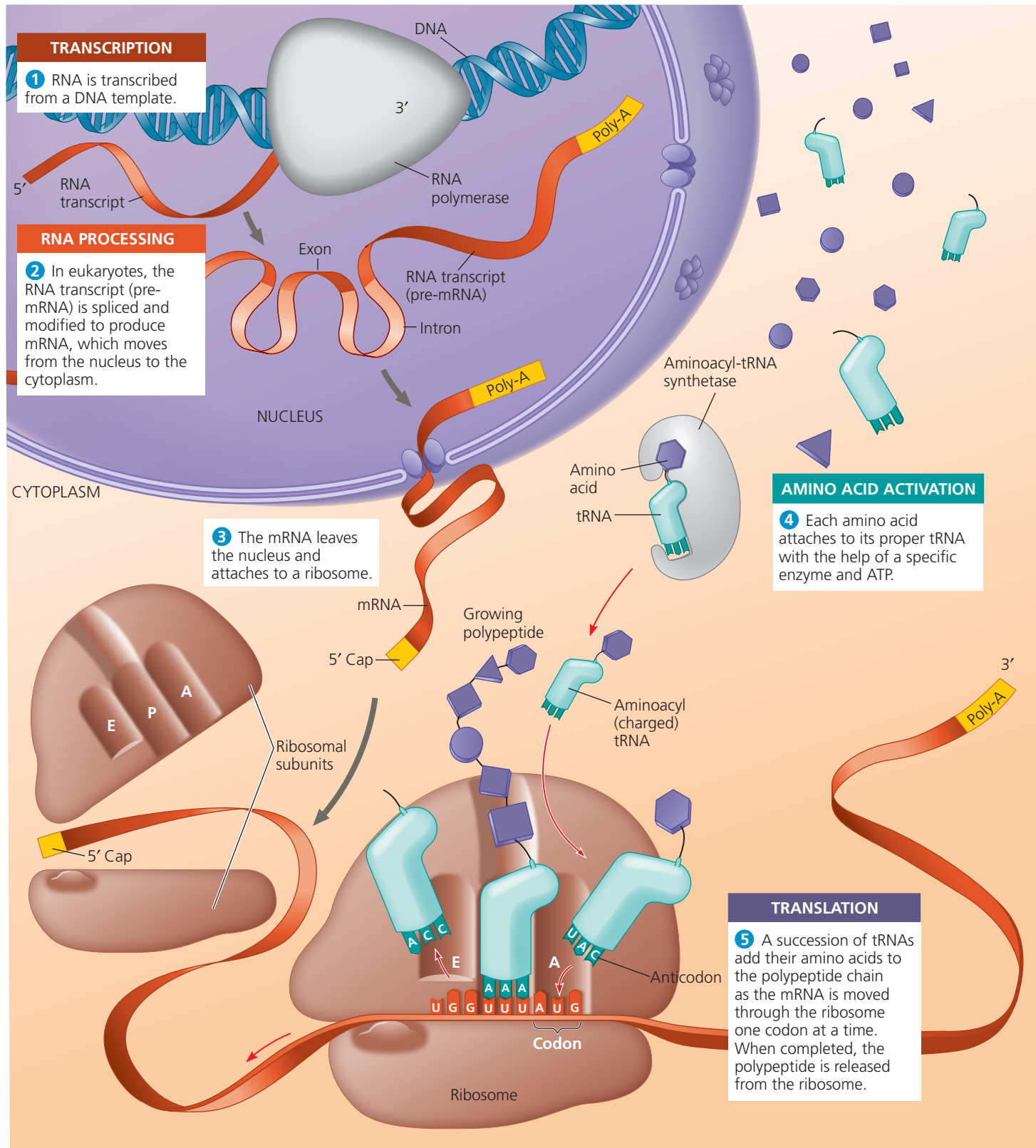
For suggested answers, see Appendix A.

▼ Figure 17.25 A summary of transcription and translation in a eukaryotic cell.

This diagram shows the path from one gene to one polypeptide. Each gene in the DNA can be transcribed repeatedly into many identical RNA molecules and each mRNA can be translated repeatedly to yield many identical polypeptide

molecules. (Also, remember that the final products of some genes are not polypeptides but RNA molecules that don't get translated, including tRNA and rRNA.) In general, the steps of transcription and translation are similar in bacterial, archaeal, and eukaryotic cells. The major difference is the occurrence of RNA

processing in the eukaryotic nucleus. Other significant differences are found in the initiation stages of both transcription and translation and in the termination of transcription. To visualize these processes in their cellular context, see Figure 7.32.



CONCEPT 17.5

Mutations of one or a few nucleotides can affect protein structure and function

Now that you have explored the process of gene expression, you are ready to understand the effects of changes to the genetic information of a cell. These changes, called **mutations**, are responsible for the huge diversity of genes found among organisms because mutations are the ultimate source of new genes. Earlier, we considered chromosomal rearrangements that affect long segments of DNA (see Figure 15.14); these are considered large-scale mutations. Here we examine small-scale mutations of one or a few nucleotide pairs, including **point mutations**, changes in a single nucleotide pair of a gene.

If a point mutation occurs in a gamete or in a cell that gives rise to gametes, it may be transmitted to offspring and to future generations. If the mutation has an adverse effect on the phenotype of a person, the mutant condition is referred to as a genetic disorder or hereditary disease. For example, we can trace the genetic basis of sickle-cell disease to the mutation of a single nucleotide pair in the gene that encodes the β -globin polypeptide of hemoglobin. The change of a single nucleotide in the DNA's template strand leads to an altered mRNA and the production of an abnormal protein (Figure 17.26; also see Figure 5.19). In individuals who are homozygous for the mutant allele, the sickling of red blood cells caused by the altered hemoglobin produces the multiple symptoms associated with sickle-cell disease (see Concept 14.4 and Figure 23.18). Another

disorder caused by a point mutation is a heart condition called familial cardiomyopathy, which is responsible for some of the tragic incidents of sudden death in young athletes. Point mutations in several genes encoding muscle proteins have been identified, any of which can lead to this disorder.

Types of Small-Scale Mutations

Let's now consider how small-scale mutations affect proteins. We should first note that many mutations occur in regions outside of protein-coding genes, and any potential effect they have on the phenotype of the organism may be subtle and hard to detect. For this reason, here we'll concentrate on mutations within protein-coding genes. Small-scale mutations within a gene can be divided into two general categories: (1) single nucleotide-pair substitutions and (2) nucleotide-pair insertions or deletions. Insertions and deletions can involve one or more nucleotide pairs.

Substitutions

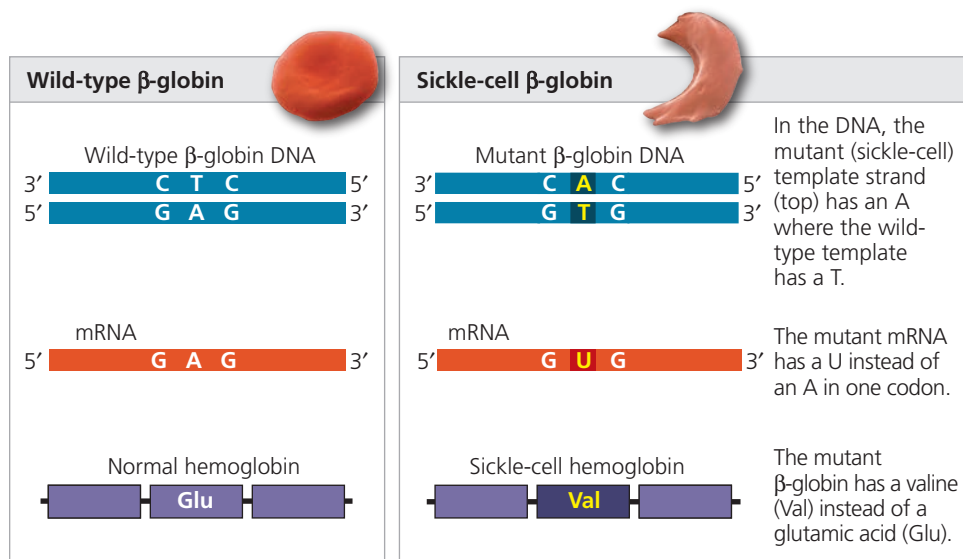
A **nucleotide-pair substitution** is the replacement of one nucleotide and its partner with another pair of nucleotides (Figure 17.27a). Some substitutions have no effect on the encoded protein, owing to the redundancy of the genetic code. For example, if 3'-CCG-5' on the template strand mutated to 3'-CCA-5', the mRNA codon that used to be GGC would become GGU, but a glycine would still be inserted at the proper location in the protein (see Figure 17.6). In other words, a change in a nucleotide pair may transform one codon into another that is translated into the same amino acid. Such a change is an example of a **silent mutation**, which has no observable

effect on the phenotype. (Silent mutations can occur outside genes as well.) Interestingly, there is evidence that some silent mutations may indirectly affect where or at what level the gene gets expressed, even though the actual protein is the same.

Substitutions that change one amino acid to another one are called **missense mutations**. Such a mutation may have little effect on the protein: The new amino acid may have properties similar to those of the amino acid it replaces, or it may be in a region of the protein where the exact sequence of amino acids is not essential to the protein's function.

However, the nucleotide-pair substitutions of greatest interest are those that cause a major change in a protein. The alteration of a single amino acid in a crucial area of a protein—such as in the part

▼ **Figure 17.26 The molecular basis of sickle-cell disease: a point mutation.** The allele that causes sickle-cell disease differs from the wild-type (normal) allele by a single DNA nucleotide pair. The micrographs are SEMs of a normal red blood cell (on the left) and a sickled red blood cell (right) from individuals homozygous for wild-type and mutant alleles, respectively.



HHMI Animation: Sickle-Cell Disease
Animation: Mutation Types



▼ **Figure 17.27 Types of small-scale mutations that affect mRNA sequence.** All but one of the types shown here also affect the amino acid sequence of the encoded polypeptide.

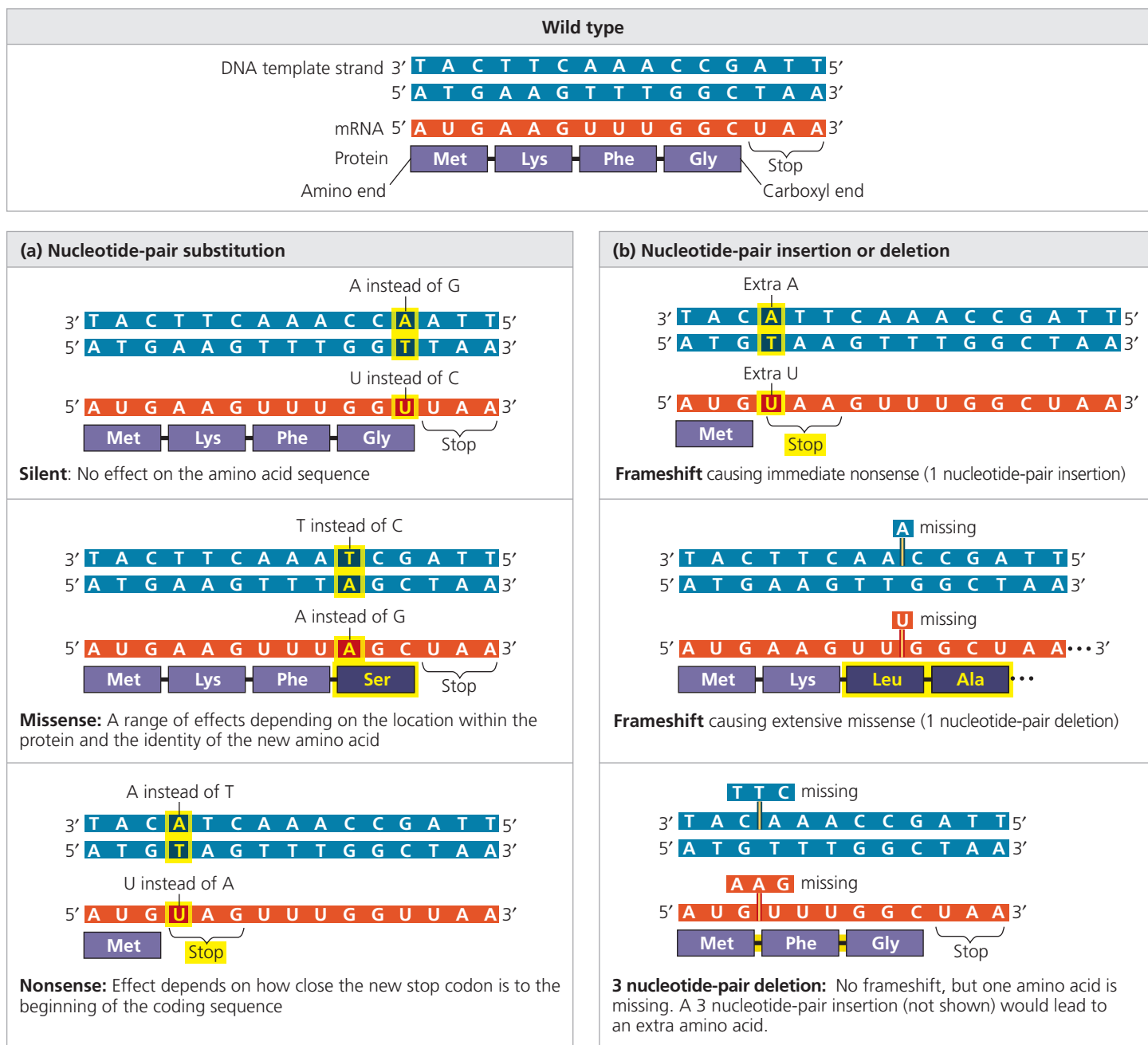


 **Figure Walkthrough**

of the β -globin subunit of hemoglobin shown in Figure 17.26 or in the active site of an enzyme as shown in Figure 6.19—can significantly alter protein activity. Occasionally, such a mutation leads to an improved protein or one with novel capabilities, but much more often such mutations are neutral or detrimental, leading to a useless or less active protein that impairs cellular function.

Substitution mutations are usually missense mutations; that is, the altered codon still codes for an amino acid and thus makes sense, although not necessarily the *right* sense.

But a point mutation can also change a codon for an amino acid into a stop codon. This is called a **nonsense mutation**, and it causes translation to be terminated prematurely; the resulting polypeptide will be shorter than the polypeptide encoded by the normal gene. Most nonsense mutations lead to nonfunctional proteins.

In the **Problem-Solving Exercise**, you'll work with a few common single nucleotide-pair substitution mutations in the gene encoding insulin, some or all of which may lead to diabetes. You will classify these mutations into one of

PROBLEM-SOLVING EXERCISE

Are insulin mutations the cause of three infants' neonatal diabetes?

Insulin is a hormone that acts as a key regulator of blood glucose level. In some cases of neonatal diabetes, the gene coding for the insulin protein has a nucleotide-pair substitution mutation that alters the protein structure enough to cause it to malfunction. How can you identify a nucleotide-pair substitution and determine its effect on the amino acid sequence?

Now that it's possible to sequence an individual's whole genome, doctors can use that DNA sequence information to diagnose diseases and identify new treatments. For example, the insulin gene sequence of a patient with neonatal diabetes can be analyzed to determine if it has a mutation and, if so, its effect.

Watch the video in the MasteringBiology Study Area to see how genome sequencing is changing medicine.



ABC News Video: Using Genome Sequencing to Diagnose Gene-Based Diseases

In this exercise, you will determine the effect of mutations present in a portion of diabetes patients' insulin gene sequences.

Your Approach Suppose you are a medical geneticist presented with three infant patients, all of whom have a nucleotide-pair substitution in their insulin gene. It is your job to analyze each mutation to figure out the effect of the mutation on the amino acid sequence of the insulin protein. To identify the mutation in each patient, you will compare his or her individual insulin complementary DNA (cDNA) sequence to that of the wild-type cDNA. (cDNA is a double-stranded DNA molecule that is based on the mRNA sequence and thus contains only the portion of a gene that is translated—introns are not included. cDNA sequences are commonly used to compare the coding regions of genes.) Identifying the codons that have been changed will tell you which, if any, amino acids are altered in the patient's insulin protein.

Your Data You will analyze the cDNA codons for amino acids 35–54 (of the 110 amino acids) of each patient's insulin protein, so the start codon (AUG) is not present. The sequences of the wild-type cDNA and the patients' cDNA are shown below, arranged in codons.

Wild-type cDNA	5'-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TTC TTC TAC ACA CCC AAG ACC-3'
Patient 1 cDNA	5'-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TGC TTC TAC ACA CCC AAG ACC-3'
Patient 2 cDNA	5'-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TCC TTC TAC ACA CCC AAG ACC-3'
Patient 3 cDNA	5'-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TTC TTG TAC ACA CCC AAG ACC-3'

Data from N. Nishi and K. Nanjo, Insulin gene mutations and diabetes, *Journal of Diabetes Investigation* 2:92–100 (2011).

- Your Analysis**
1. Comparing each patient's cDNA sequence to the wild-type cDNA sequence, circle the codons where a nucleotide-pair substitution mutation has occurred.
 2. Use a codon table (see Figure 17.6) to identify the amino acid that will be made by the codon with the mutation in each patient's insulin sequence, and compare it to the amino acid made by the codon in the corresponding wild-type sequence. As is standard practice with DNA sequences, the cDNA *coding* (nontemplate) strand has been provided, so to convert it to mRNA for use with the codon table, you just need to change T to U. Classify each patient's nucleotide-pair substitution mutation: Is it a silent, missense, or nonsense mutation? Explain, for each answer.
 3. Compare the structure of the amino acid you identified in each patient's insulin sequence to that of the corresponding amino acid in the wild-type insulin sequence (see Figure 5.14). Given that each patient has neonatal diabetes, discuss how the change of amino acid in each might have affected the insulin protein and thus resulted in the disease.



Instructors: A version of this Problem-Solving Exercise can be assigned in MasteringBiology. Or a more extensive investigation called "Solve It: Which Insulin Mutations May Result in Disease?" can be assigned.

the types we just described and characterize the change in amino acid sequence.

Insertions and Deletions

Insertions and **deletions** are additions or losses of nucleotide pairs in a gene (Figure 17.27b). These mutations have a disastrous effect on the resulting protein more often than

substitutions do. Insertion or deletion of nucleotides may alter the reading frame of the genetic message, the triplet grouping of nucleotides on the mRNA that is read during translation. Such a mutation, called a **frameshift mutation**, occurs whenever the number of nucleotides inserted or deleted is not a multiple of three. All nucleotides downstream of the deletion or insertion will be improperly grouped into codons; the result will be

extensive missense mutations, usually ending sooner or later in a nonsense mutation that leads to premature termination. Unless the frameshift is very near the end of the gene, the protein is almost certain to be nonfunctional. Insertions and deletions also occur outside of coding regions; these are not called frameshift mutations, but can have effects on the phenotype—for instance, they can affect how a gene is expressed.

New Mutations and Mutagens

Mutations can arise in a number of ways. Errors during DNA replication or recombination can lead to nucleotide-pair substitutions, insertions, or deletions, as well as to mutations affecting longer stretches of DNA. If an incorrect nucleotide is added to a growing chain during replication, for example, the base on that nucleotide will then be mismatched with the nucleotide base on the other strand. In many cases, the error will be corrected by DNA proofreading and repair systems (see Concept 16.2). Otherwise, the incorrect base will be used as a template in the next round of replication, resulting in a mutation. Such mutations are called *spontaneous mutations*. It is difficult to calculate the rate at which such mutations occur. Rough estimates have been made of the rate of mutation during DNA replication for both *E. coli* and eukaryotes, and the numbers are similar: About one nucleotide in every 10^{10} is altered, and the change is passed on to the next generation of cells.

A number of physical and chemical agents, called **mutagens**, interact with DNA in ways that cause mutations. In the 1920s, Hermann Muller discovered that X-rays caused genetic changes in fruit flies, and he used X-rays to make *Drosophila* mutants for his genetic studies. But he also recognized an alarming implication of his discovery: X-rays and other forms of high-energy radiation pose hazards to the genetic material of people as well as laboratory organisms. Mutagenic radiation, a physical mutagen, includes ultraviolet (UV) light, which can cause disruptive thymine dimers in DNA (see Figure 16.19).

Chemical mutagens fall into several categories. Nucleotide analogs are chemicals similar to normal DNA nucleotides but that pair incorrectly during DNA replication. Other chemical mutagens interfere with correct DNA replication by inserting themselves into the DNA and distorting the double helix. Still other mutagens cause chemical changes in bases that change their pairing properties.

Researchers have developed a variety of methods to test the mutagenic activity of chemicals. A major application of these tests is the preliminary screening of chemicals to identify those that may cause cancer. This approach makes sense because most carcinogens (cancer-causing chemicals) are mutagenic, and conversely, most mutagens are carcinogenic.

What Is a Gene? Revisiting the Question

Our definition of a gene has evolved over the past few chapters, as it has through the history of genetics. We began with the Mendelian concept of a gene as a discrete unit of inheritance

that affects a phenotypic character (Chapter 14). We saw that Morgan and his colleagues assigned such genes to specific loci on chromosomes (Chapter 15). We went on to view a gene as a region of specific nucleotide sequence along the length of the DNA molecule of a chromosome (Chapter 16). Finally, in this chapter, we have considered a functional definition of a gene as a DNA sequence that codes for a specific polypeptide chain or a functional RNA molecule, such as a tRNA. All these definitions are useful, depending on the context in which genes are being studied.

We have noted that merely saying a gene codes for a polypeptide is an oversimplification. Most eukaryotic genes contain noncoding segments (such as introns), so large portions of these genes have no corresponding segments in polypeptides. Molecular biologists also often include promoters and certain other regulatory regions of DNA within the boundaries of a gene. These DNA sequences are not transcribed, but they can be considered part of the functional gene because they must be present for transcription to occur. Our definition of a gene must also be broad enough to include the DNA that is transcribed into rRNA, tRNA, and other RNAs that are not translated. These genes have no polypeptide products but play crucial roles in the cell. Thus, we arrive at the following definition: *A gene is a region of DNA that can be expressed to produce a final functional product that is either a polypeptide or an RNA molecule.*

When considering phenotypes, however, it is often useful to start by focusing on genes that code for polypeptides. In this chapter, you have learned in molecular terms how a typical gene is expressed—by transcription into RNA and then translation into a polypeptide that forms a protein of specific structure and function. Proteins, in turn, bring about an organism's observable phenotype.

A given type of cell expresses only a subset of its genes. This is an essential feature in multicellular organisms: You'd be in trouble if the lens cells in your eyes started expressing the genes for hair proteins, which are normally expressed only in hair follicle cells! Gene expression is precisely regulated, which we'll explore in the next chapter, beginning with the simpler case of bacteria and continuing with eukaryotes.

CONCEPT CHECK 17.5

1. What happens when one nucleotide pair is lost from the middle of the coding sequence of a gene?
2. **MAKE CONNECTIONS** > Individuals heterozygous for the sickle-cell allele are generally healthy but show phenotypic effects of the allele under some circumstances (see Figure 14.17). Explain in terms of gene expression.
3. **WHAT IF? DRAW IT** > The template strand of a gene includes this sequence:
3'-TACTTGTCCGATATC-5'. It is mutated to
3'-TACTTGTCCAATATC-5'. For both wild-type and mutant sequences, draw the double-stranded DNA, the resulting mRNA, and the amino acid sequence each encodes. What is the effect of the mutation on the amino acid sequence?

For suggested answers, see Appendix A.

17 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 17.1

Genes specify proteins via transcription and translation (pp. 386–392)



VOCAB
SELF-QUIZ
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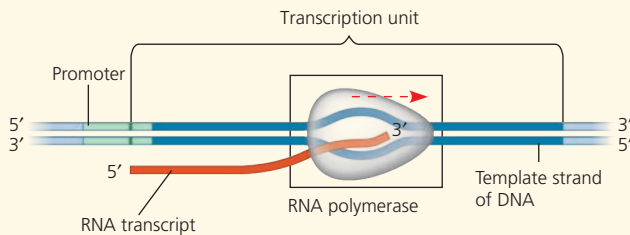
- Beadle and Tatum's studies of mutant strains of *Neurospora* led to the one gene–one polypeptide hypothesis. During **gene expression**, the information encoded in genes is used to make specific polypeptide chains (enzymes and other proteins) or RNA molecules.
- **Transcription** is the synthesis of RNA complementary to a **template strand** of DNA. **Translation** is the synthesis of a polypeptide whose amino acid sequence is specified by the nucleotide sequence in **messenger RNA (mRNA)**.
- Genetic information is encoded as a sequence of nonoverlapping nucleotide triplets, or **codons**. A codon in mRNA either is translated into an amino acid (61 of the 64 codons) or serves as a stop signal (3 codons). Codons must be read in the correct **reading frame**.

? Describe the process of gene expression, by which a gene affects the phenotype of an organism.

CONCEPT 17.2

Transcription is the DNA-directed synthesis of RNA: a closer look (pp. 392–394)

- RNA synthesis is catalyzed by **RNA polymerase**, which links together RNA nucleotides complementary to a DNA template strand. This process follows the same base-pairing rules as DNA replication, except that in RNA, uracil substitutes for thymine.



- The three stages of transcription are initiation, elongation, and termination. A **promoter**, often including a **TATA box** in eukaryotes, establishes where RNA synthesis is initiated. **Transcription factors** help eukaryotic RNA polymerase recognize promoter sequences, forming a **transcription initiation complex**. Termination differs in bacteria and eukaryotes.

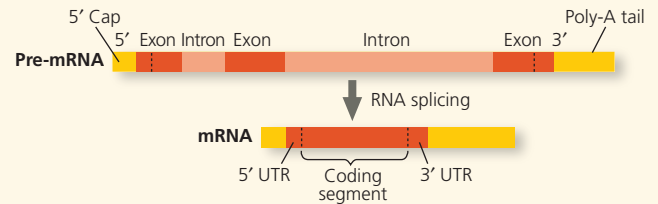
? What are the similarities and differences in the initiation of gene transcription in bacteria and eukaryotes?

CONCEPT 17.3

Eukaryotic cells modify RNA after transcription (pp. 395–397)

- Eukaryotic mRNAs undergo **RNA processing**, which includes RNA splicing, the addition of a modified nucleotide **5' cap** to the 5' end, and the addition of a **poly-A tail** to the 3' end. The processed mRNA includes an untranslated region (5' UTR or 3' UTR) at each end of the coding segment.

- Most eukaryotic genes are split into segments: They have **introns** interspersed among the **exons** (the regions included in the mRNA). In **RNA splicing**, introns are removed and exons joined. RNA splicing is typically carried out by **spliceosomes**, but in some cases, RNA alone catalyzes its own splicing. The catalytic ability of some RNA molecules, called **ribozymes**, derives from the inherent properties of RNA. The presence of introns allows for **alternative RNA splicing**.

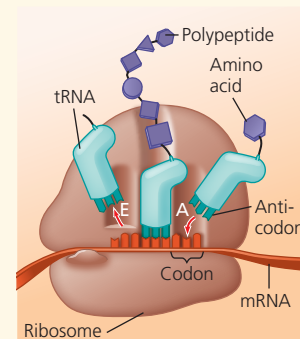


? What function do the 5' cap and the poly-A tail serve on a eukaryotic mRNA?

CONCEPT 17.4

Translation is the RNA-directed synthesis of a polypeptide: a closer look (pp. 397–406)

- A cell translates an mRNA message into protein using **transfer RNAs (tRNAs)**. After being bound to a specific amino acid by an **aminoacyl-tRNA synthetase**, a tRNA lines up via its **anticodon** at the complementary codon on mRNA. A **ribosome**, made up of **ribosomal RNAs (rRNAs)** and proteins, facilitates this coupling with binding sites for mRNA and tRNA.
- Ribosomes coordinate the three stages of translation: initiation, elongation, and termination. The formation of peptide bonds between amino acids is catalyzed by rRNAs as tRNAs move through the **A** and **P** sites and exit through the **E** site.



- After translation, during protein processing, proteins may be modified by cleavage or by attachment of sugars, lipids, phosphates, or other chemical groups.
- Free ribosomes in the cytosol initiate synthesis of all proteins, but proteins with a **signal peptide** are synthesized on the ER.
- A gene can be transcribed by multiple RNA polymerases simultaneously. Also, a single mRNA molecule can be translated simultaneously by a number of ribosomes, forming a **polyribosome**. In bacteria, these processes are coupled, but in eukaryotes they are separated in space and time by the nuclear membrane.

? Describe how tRNAs function in the context of the ribosome in building a polypeptide.

CONCEPT 17.5

Mutations of one or a few nucleotides can affect protein structure and function (pp. 407–410)

- Small-scale **mutations** include **point mutations**, changes in one DNA nucleotide pair, which may lead to production of non-functional proteins. **Nucleotide-pair substitutions** can cause **missense** or **nonsense mutations**. Nucleotide-pair **insertions** or **deletions** may produce **frameshift mutations**.
- Spontaneous mutations can occur during DNA replication and recombination. Chemical and physical **mutagens** cause DNA damage that can alter genes.

? What will be the results of chemically modifying one nucleotide base of a gene? What role is played by DNA repair systems in the cell?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. Would the coupling of the processes shown in Figure 17.24 be found in a eukaryotic cell? Explain why or why not.

9. Complete the following table:

Type of RNA	Functions
Messenger RNA (mRNA)	
Transfer RNA (tRNA)	
	In a ribosome, plays a structural role; as a ribozyme, plays a catalytic role (catalyzes peptide bond formation)
Primary transcript	
Small RNAs in the spliceosome	



PRACTICE TEST
goo.gl/iAsVgL

10. **EVOLUTION CONNECTION** Most amino acids are coded for by a set of similar codons (see Figure 17.6). Propose at least one evolutionary explanation to account for this pattern.

11. **SCIENTIFIC INQUIRY** During the sequence analysis of a eukaryotic gene, you discover that it contains a restriction site for the enzyme NotI. This site is 1500 bp away from the restriction site for the enzyme XbaI. When the cDNA for the gene is treated with both the enzymes, the two sites are found to be only 110 bp apart. What is the most likely reason for this result?
12. **WRITE ABOUT A THEME: INFORMATION** Evolution accounts for the unity and diversity of life, and the continuity of life is based on heritable information in the form of DNA. In a short essay (100–150 words), discuss how the fidelity with which DNA is inherited is related to the processes of evolution. (Review the discussion of proofreading and DNA repair in Concept 16.2.)

13. SYNTHESIZE YOUR KNOWLEDGE



Some mutations result in proteins that function well at one temperature but are nonfunctional at a different (usually higher) temperature. Siamese cats have such a “temperature-sensitive” mutation in a gene encoding an enzyme that makes dark pigment in the fur. The mutation results in the breed’s distinctive point markings and lighter body color (see the photo). Using this information and what you learned in the chapter, explain the pattern of the cat’s fur pigmentation.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Control of Gene Expression

18



▲ **Figure 18.1** How can this fish's eyes see equally well in both air and water?

KEY CONCEPTS

- 18.1** Bacteria often respond to environmental change by regulating transcription
- 18.2** Eukaryotic gene expression is regulated at many stages
- 18.3** Noncoding RNAs play multiple roles in controlling gene expression
- 18.4** A program of differential gene expression leads to the different cell types in a multicellular organism
- 18.5** Cancer results from genetic changes that affect cell cycle control



Beauty in the Eye of the Beholder

The fish in **Figure 18.1** is keeping an eye out for predators—or, more precisely, both halves of each eye! *Anableps anableps*, commonly known as “cuatro ojos” (“four eyes”), glides through freshwater lakes and ponds in Central and South America with the upper half of each eye protruding from the water. The eye’s upper half is particularly well-suited for aerial vision and the lower half for aquatic vision. The molecular basis of this specialization has recently been revealed: The cells of the two parts of the eye express a slightly different set of genes involved in vision, even though these two groups of cells are quite similar and contain identical genomes. What is the biological mechanism underlying the difference in gene expression that makes this remarkable feat possible?

A hallmark of prokaryotic and eukaryotic cells alike—from a bacterium to the cells of a fish—is their intricate and precise regulation of gene expression. In this chapter, we first explore how bacteria regulate expression of their genes in response to different environmental conditions. We then examine the general mechanisms by which eukaryotes regulate gene expression, including the many roles played by RNA molecules. In the final two sections, we explore the role of gene regulation in both embryonic development, as the ultimate example of proper gene regulation, and cancer, as an illustration of what happens when regulation goes awry. Orchestrating proper gene expression by all cells is crucial to the functions of life.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

CONCEPT 18.1

Bacteria often respond to environmental change by regulating transcription

Bacterial cells that can conserve resources and energy have a selective advantage over cells that are unable to do so. Thus, natural selection has favored bacteria that express only the genes whose products are needed by the cell.

Consider, for instance, an individual *Escherichia coli* (*E. coli*) cell living in the erratic environment of a human colon, dependent for its nutrients on the whimsical eating habits of its host. If the environment is lacking in the amino acid tryptophan, which the bacterium needs to survive, the cell responds by activating a metabolic pathway that makes tryptophan from another compound. If the human host later eats a tryptophan-rich meal, the bacterial cell stops producing tryptophan, thus avoiding wasting resources to produce a substance that is readily available from the surrounding solution.

A metabolic pathway can be controlled on two levels, as shown for the synthesis of tryptophan in **Figure 18.2**. First, cells can adjust the activity of enzymes already present. This is a fairly rapid response, which relies on the sensitivity of many enzymes to chemical cues that increase or decrease their catalytic activity (see Concept 6.5). The activity of the first enzyme in the pathway is inhibited by the pathway's end product—tryptophan, in this case (**Figure 18.2a**). Thus, if tryptophan accumulates in a cell, it shuts down the synthesis of more tryptophan by inhibiting enzyme activity. Such *feedback inhibition*, typical of anabolic (biosynthetic) pathways, allows a cell to adapt to short-term fluctuations in the supply of a substance it needs (see Figure 6.21).

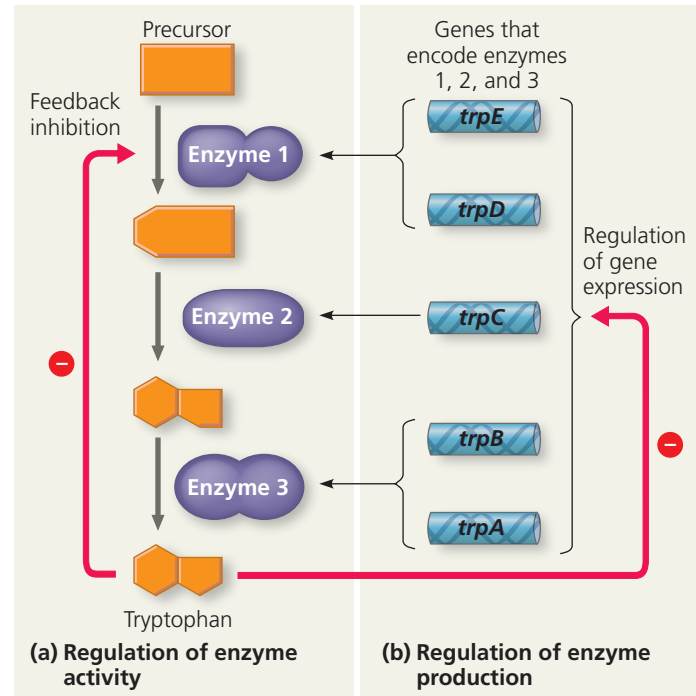
Second, cells can adjust the production level of certain enzymes; that is, they can regulate the expression of the genes encoding the enzymes. If, in our example, the environment provides all the tryptophan the cell needs, the cell stops making the enzymes that catalyze the synthesis of tryptophan (**Figure 18.2b**). In this case, the control of enzyme production occurs at the level of transcription, the synthesis of messenger RNA from the genes that code for these enzymes.

Regulation of the tryptophan synthesis pathway is just one example of how bacteria tune their metabolism to changing environments. Many genes of the bacterial genome are switched on or off by changes in the metabolic status of the cell. One basic mechanism for this control of gene expression in bacteria, described as the *operon model*, was discovered in 1961 by François Jacob and Jacques Monod at the Pasteur Institute in Paris. Let's see what an operon is and how it works.

Operons: The Basic Concept

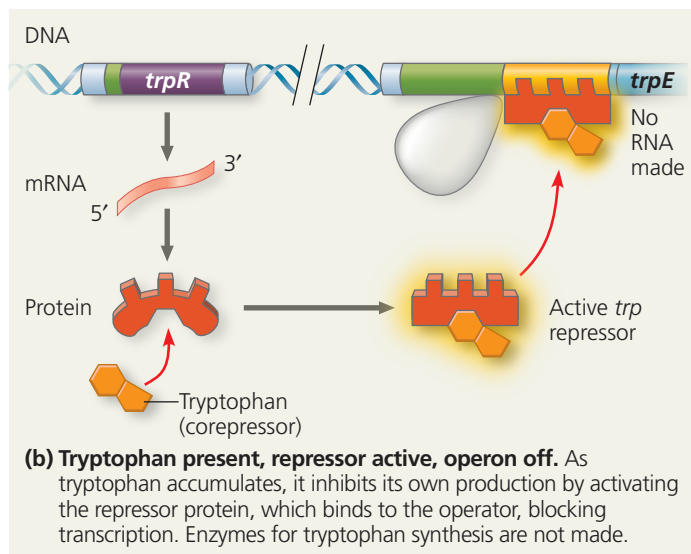
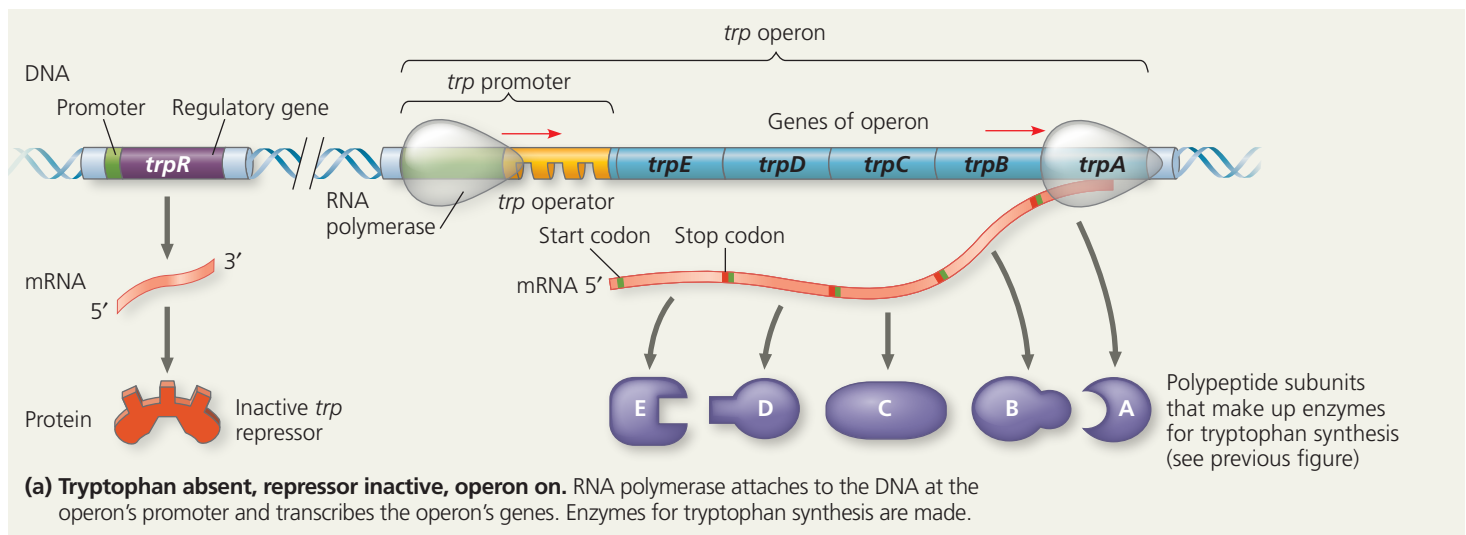
E. coli synthesizes the amino acid tryptophan from a precursor molecule in the three-step pathway shown in Figure 18.2.

▼ **Figure 18.2 Regulation of a metabolic pathway.** In the pathway for tryptophan synthesis, an abundance of tryptophan can both (a) inhibit the activity of the first enzyme in the pathway (feedback inhibition), a rapid response, and (b) repress expression of the genes encoding all subunits of the enzymes in the pathway, a longer-term response. Genes *trpE* and *trpD* encode the two subunits of enzyme 1, and genes *trpB* and *trpA* encode the two subunits of enzyme 3. (The genes were named before the order in which they functioned in the pathway was determined.) The symbol  stands for inhibition.



Each reaction in the pathway is catalyzed by a specific enzyme, and the five genes that code for the subunits of these enzymes are clustered together on the bacterial chromosome. A single promoter serves all five genes, which together constitute a transcription unit. (Recall that a promoter is a site where RNA polymerase can bind to DNA and begin transcription; see Figure 17.8.) Thus, transcription gives rise to one long mRNA molecule that codes for the five polypeptides making up the enzymes in the tryptophan pathway (**Figure 18.3a**). The cell can translate this one mRNA into five separate polypeptides because the mRNA is punctuated with start and stop codons that signal where the coding sequence for each polypeptide begins and ends.

A key advantage of grouping genes of related function into one transcription unit is that a single "on-off switch" can control the whole cluster of functionally related genes; in other words, these genes are *coordinately controlled*. When an *E. coli* cell must make tryptophan for itself because its surrounding environment lacks this amino acid, all the enzymes for the metabolic pathway are synthesized at the same time. The on-off switch is a segment of DNA called an **operator**. Both its location and name suit its function: Positioned within the promoter or, in some cases, between the promoter and the enzyme-coding genes, the operator controls the access



▲ Figure 18.3 The *trp* operon in *E. coli*: regulated synthesis of repressible enzymes. Tryptophan is an amino acid produced by an anabolic pathway catalyzed by three enzymes (see Figure 18.2).

(a) The five genes encoding the polypeptide subunits of the enzymes in this pathway are grouped, along with a promoter, into the *trp* operon. The *trp* operator (the repressor binding site) is located within the *trp* promoter (the RNA polymerase binding site). **(b)** Accumulation of tryptophan, the end product of the pathway, represses transcription of the *trp* operon, thus blocking synthesis of all the enzymes in the pathway and shutting down tryptophan production.

VISUAL SKILLS ▶ Describe what happens to the *trp* operon as the cell uses up its store of tryptophan.

of RNA polymerase to the genes. Together, the operator, the promoter, and the genes they control—the entire stretch of DNA required for enzyme production for the tryptophan pathway—constitute an **operon**. The *trp* operon (*trp* for tryptophan) is one of many operons in the *E. coli* genome (see Figure 18.3a).

If the operator is the operon's switch for controlling transcription, how does this switch work? By itself, the *trp* operon is turned on; that is, RNA polymerase can bind to the promoter and transcribe the genes of the operon. The *trp* operon can be switched off by a protein that is called the *trp* repressor. A **repressor** binds to the operator and blocks attachment of RNA polymerase to the promoter, preventing transcription of the genes (**Figure 18.3b**). A repressor protein is specific for the operator of a particular operon. For example, the *trp* repressor, which switches off the *trp* operon by binding to the *trp* operator, has no effect on other operons in the *E. coli* genome.

A repressor protein is encoded by a **regulatory gene**—in this case, a gene called *trpR*; *trpR* is located some distance from the *trp* operon and has its own promoter. Regulatory genes are expressed continuously, although at a low rate, and a

few *trp* repressor molecules are always present in *E. coli* cells. Why, then, is the *trp* operon not switched off permanently? First, the binding of repressors to operators is reversible. An operator alternates between two states: one with the repressor bound and one without the repressor bound. The relative duration of the repressor-bound state is higher when more active repressor molecules are present. Second, the *trp* repressor, like most regulatory proteins, is an allosteric protein, with two alternative shapes: active and inactive (see Figure 6.20). The *trp* repressor is synthesized in the inactive form, which has little affinity for the *trp* operator. Only when a tryptophan molecule binds to the *trp* repressor at an allosteric site does the repressor protein change to the active form that can attach to the operator, turning the operon off.

Tryptophan functions in this system as a **corepressor**, a small molecule that cooperates with a repressor protein to switch an operon off. As tryptophan accumulates, more tryptophan molecules associate with *trp* repressor molecules, which can then bind to the *trp* operator and shut down production of the tryptophan pathway enzymes. If the cell's tryptophan level drops, many fewer *trp* repressor proteins would have tryptophan bound, rendering them inactive; they would dissociate from the operator, allowing transcription of the operon's genes to resume. The *trp* operon is one example of how gene expression can respond to changes in the cell's internal and external environment.

Repressible and Inducible Operons: Two Types of Negative Gene Regulation

The *trp* operon is said to be a *repressible operon* because its transcription is usually on but can be inhibited (repressed) when a specific small molecule (in this case, tryptophan) binds allosterically to a regulatory protein. In contrast, an *inducible operon* is usually off but can be stimulated (induced) to be on when a specific small molecule interacts with a different regulatory protein. The classic example of an inducible operon is the *lac* operon (*lac* stands for “lactose”).

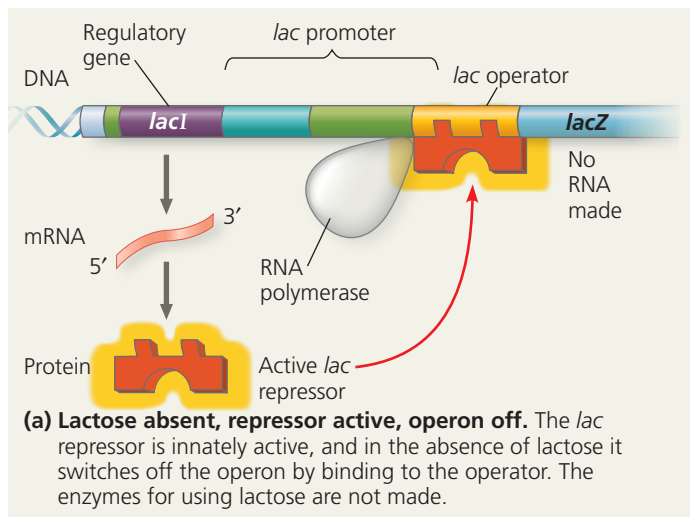
The disaccharide lactose (milk sugar) is available to *E. coli* in the human colon if the host drinks milk or eats a dairy product. Lactose metabolism begins with hydrolysis of the disaccharide into its component monosaccharides (glucose and galactose), a reaction catalyzed by the enzyme β -galactosidase. Only a few molecules of this enzyme are present in an *E. coli* cell growing in the absence of lactose. If lactose is added to the bacterium’s environment, however, the number of β -galactosidase

molecules in the cell increases 1,000-fold within about 15 minutes. How can a cell ramp up enzyme production this quickly?

The gene for β -galactosidase (*lacZ*) is part of the *lac* operon, which includes two other genes coding for enzymes that function in the use of lactose (**Figure 18.4**). The entire transcription unit is under the command of one main operator and promoter. The regulatory gene, *lacI*, located outside the *lac* operon, codes for an allosteric repressor protein that can switch off the *lac* operon by binding to the *lac* operator. So far, this sounds just like regulation of the *trp* operon, but there is one important difference. Recall that the *trp* repressor protein is inactive by itself and requires tryptophan as a corepressor in order to bind to the operator. The *lac* repressor, in contrast, is active by itself, binding to the operator and switching the *lac* operon off. In this case, a specific small molecule, called an **inducer**, *inactivates* the repressor.

For the *lac* operon, the inducer is allolactose, an isomer of lactose formed in small amounts from lactose that enters the cell. In the absence of lactose (and hence allolactose), the *lac* repressor is in its active shape and binds to the operator; thus, the genes of the *lac* operon are silenced (**Figure 18.4a**). If lactose is added to the cell’s surroundings, allolactose binds to the *lac* repressor and alters its shape so the repressor can no longer bind to the operator. Without the *lac* repressor bound, the *lac* operon is transcribed into mRNA, and the enzymes for using lactose are made (**Figure 18.4b**).

In the context of gene regulation, the enzymes of the lactose pathway are referred to as *inducible enzymes* because their synthesis is induced by a chemical signal (allolactose, in this case). Analogously, the enzymes for tryptophan synthesis are said to be repressible. *Repressible enzymes* generally function in anabolic pathways, which synthesize essential end products from raw materials (precursors). By suspending production



Animation: The *lac* Operon in *E. coli*

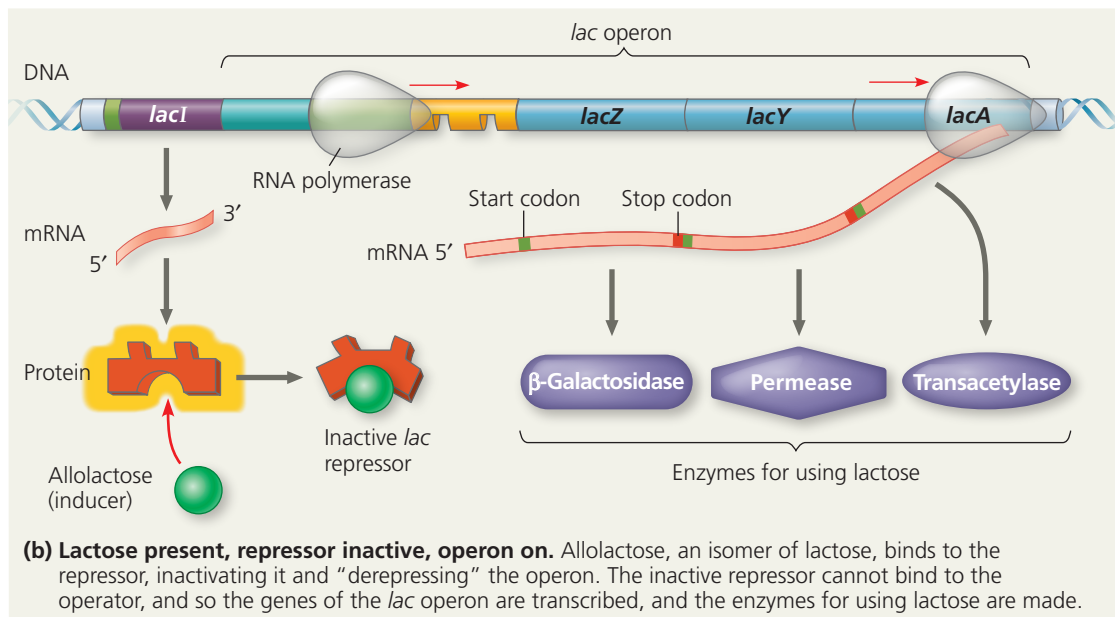


Figure 18.4 The *lac* operon in *E. coli*: regulated synthesis of inducible enzymes. *E. coli* uses three enzymes to take up and metabolize lactose, the genes for which are clustered in the *lac* operon. The first gene, *lacZ*, codes for β -galactosidase, which hydrolyzes lactose to glucose and galactose. The second, *lacY*, codes for a permease, the membrane protein that transports lactose into the cell. The third, *lacA*, codes for transacetylase, an enzyme that detoxifies other molecules entering the cell via the permease. Unusually, the gene for the *lac* repressor, *lacI*, is adjacent to the *lac* operon. The function of the teal region within the promoter will be revealed in Figure 18.5.

of an end product when it is already present in sufficient quantity, the cell can allocate its organic precursors and energy for other uses. In contrast, inducible enzymes usually function in catabolic pathways, which break down a nutrient to simpler molecules. By producing the appropriate enzymes only when the nutrient is available, the cell avoids wasting energy and precursors making proteins that are not needed.

Regulation of both the *trp* and *lac* operons involves the *negative* control of genes because the operons are switched off by the active form of their respective repressor protein. It may be easier to see this for the *trp* operon, but it is also true for the *lac* operon. In the case of the *lac* operon, allolactose induces enzyme synthesis not by directly activating the *lac* operon, but by freeing it from the negative effect of the repressor (see Figure 18.4b). Gene regulation is said to be *positive* only when a regulatory protein interacts directly with the genome to switch transcription on.

Positive Gene Regulation

When glucose and lactose are both present in its environment, *E. coli* preferentially uses glucose. The enzymes for glucose breakdown in glycolysis (see Figure 10.9) are continually present. Only when lactose is present *and* glucose is in short supply does *E. coli* use lactose as an energy source, and only then does it synthesize appreciable quantities of the enzymes for lactose breakdown.

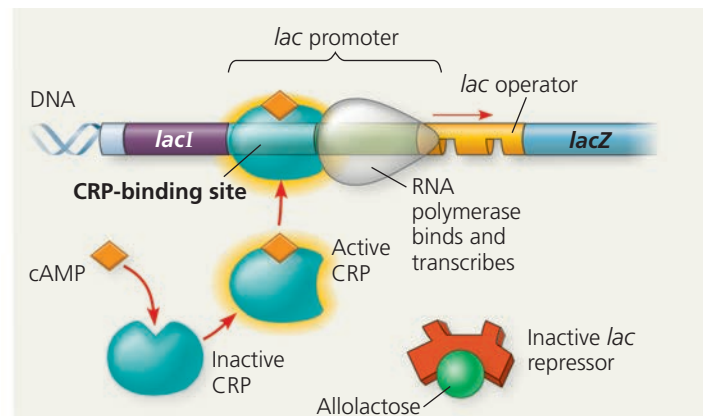
How does the *E. coli* cell sense the glucose concentration and relay this information to the *lac* operon? Again, the mechanism depends on the interaction of an allosteric regulatory protein with a small organic molecule, **cyclic AMP (cAMP)** in this case, which accumulates when glucose is scarce (see Figure 9.11 for the structure of cAMP). The regulatory protein, called *cAMP receptor protein* (CRP), is an **activator**, a protein that binds to DNA and stimulates transcription of a gene. When cAMP binds to this regulatory protein, CRP assumes its active shape and can attach to a specific site at the upstream end of the *lac* promoter (Figure 18.5a). This attachment increases the affinity of RNA polymerase for the *lac* promoter, which is actually rather low even when no *lac* repressor is bound to the operator. By facilitating the binding of RNA polymerase to the promoter and thereby increasing the rate of transcription of the *lac* operon, the attachment of CRP to the promoter directly stimulates gene expression. Therefore, this mechanism qualifies as positive regulation.

If the amount of glucose in the cell increases, the cAMP concentration falls, and without cAMP, CRP detaches from the *lac* operon. Because CRP is inactive, RNA polymerase binds less efficiently to the promoter, and transcription of the *lac* operon proceeds only at a low level, even when lactose is present (Figure 18.5b). Thus, the *lac* operon is under dual control: negative control by the *lac* repressor and positive control by CRP. The state of the *lac* repressor (with allolactose bound or without it) determines whether or not transcription of the *lac* operon's genes occurs at all; the state of CRP (with bound

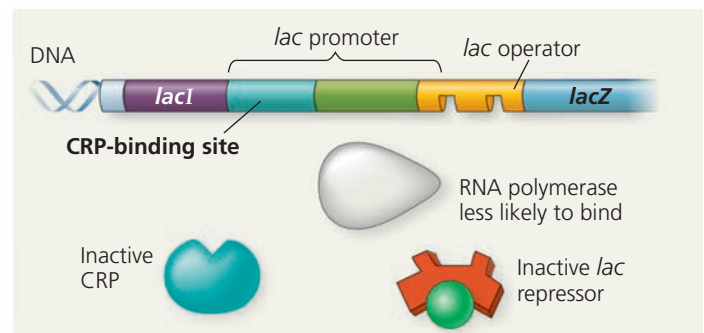
cAMP or without it) controls the *rate* of transcription if the operon is repressor-free. It is as though the operon has both an on-off switch and a volume control.

In addition to regulating the *lac* operon, CRP helps regulate other operons that encode enzymes used in catabolic pathways. All told, it may affect the expression of more than 100 genes in *E. coli*. When glucose is plentiful and CRP is inactive, the synthesis of enzymes that catabolize compounds other than glucose generally slows down. The ability to catabolize other compounds, such as lactose, enables a cell deprived of glucose to survive. The compounds present in any given cell at the moment determine which operons are switched on—the result of simple interactions of activator and repressor proteins with the promoters of the genes in question.

✓ **Figure 18.5 Positive control of the *lac* operon by cAMP receptor protein (CRP).** RNA polymerase has high affinity for the *lac* promoter only when CRP is bound to a DNA site at the upstream end of the promoter. CRP, in turn, attaches to its DNA site only when associated with cyclic AMP (cAMP), whose concentration in the cell rises when the glucose concentration falls. Thus, when glucose is present, even if lactose is also available, the cell preferentially catabolizes glucose and makes very little of the enzymes for using lactose.



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized. If glucose is scarce, the high level of cAMP activates CRP, which binds to the promoter and increases RNA polymerase binding there. The *lac* operon produces large amounts of mRNA coding for the enzymes that the cell needs for use of lactose.



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized. When glucose is present, cAMP is scarce, and CRP is unable to stimulate transcription at a significant rate, even though no repressor is bound.

CONCEPT CHECK 18.1

1. How does binding of the *trp* corepressor to the *trp* repressor alter repressor function and transcription? What about the binding of the *lac* inducer to the *lac* repressor?
2. Describe the binding of RNA polymerase, repressors, and activators to the *lac* operon when both lactose and glucose are scarce. What is the effect of these scarcities on transcription of the *lac* operon?
3. **WHAT IF? >** A certain mutation in *E. coli* changes the *lac* operator so that the active repressor cannot bind. How would this affect the cell's production of β -galactosidase?

For suggested answers, see Appendix A.

CONCEPT 18.2

Eukaryotic gene expression is regulated at many stages

All organisms, whether prokaryotes or eukaryotes, must regulate which genes are expressed at any given time. Both unicellular organisms and the cells of multicellular organisms continually turn genes on and off in response to signals from their external and internal environments. Regulation of gene expression is also essential for cell specialization in multicellular organisms, which are made up of different types of cells. To perform its own distinct role, each cell type must maintain a specific program of gene expression in which certain genes are expressed and others are not.

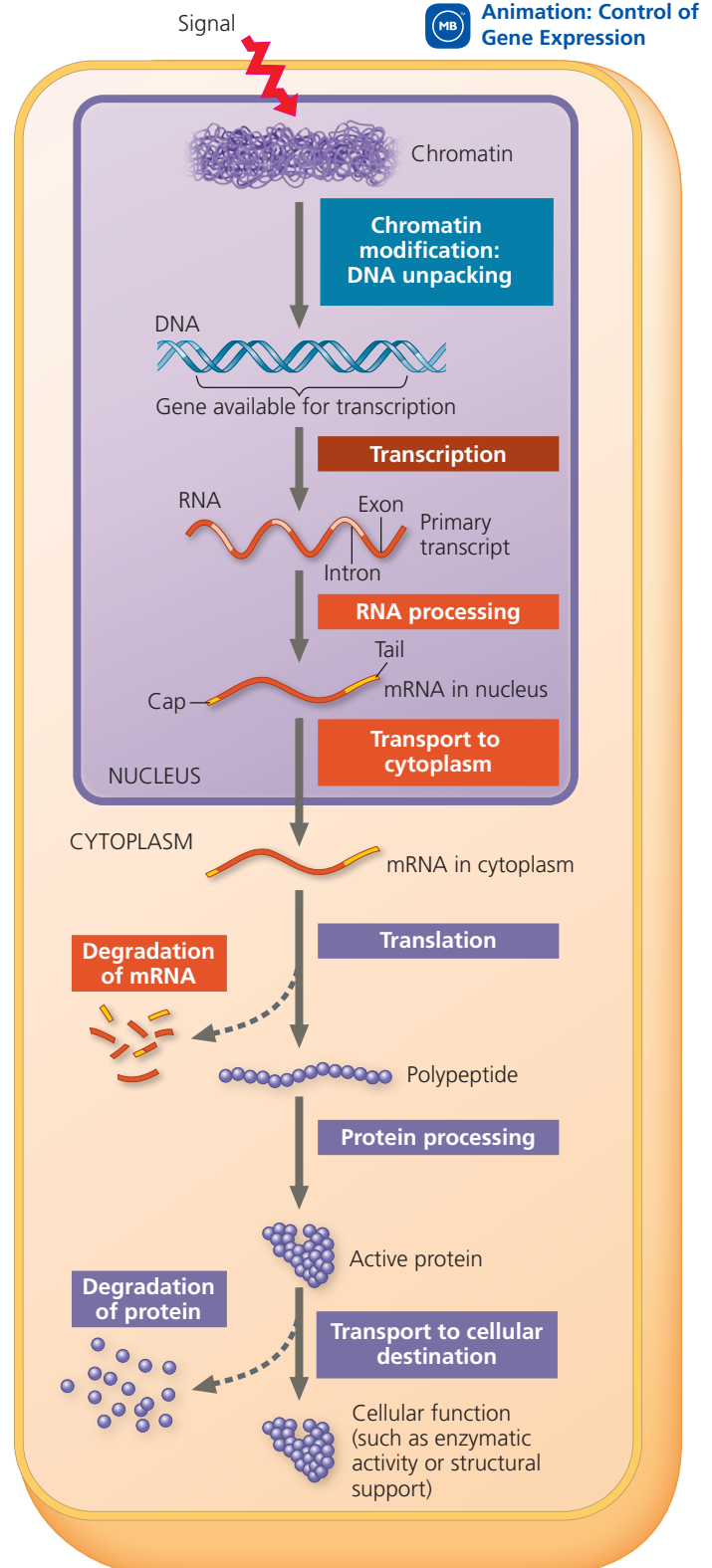
Differential Gene Expression

A typical human cell might express about 20% of its protein-coding genes at any given time. Highly differentiated cells, such as muscle or nerve cells, express an even smaller fraction of their genes. Almost all the cells in a multicellular organism contain an identical genome. (Cells of the immune system are one exception, as you will see in Chapter 47.) A subset of genes is expressed in each cell type; some of these are “house-keeping” genes, expressed by many cell types, while others are unique to that cell type. The uniquely expressed genes allow these cells to carry out their specific function. The differences between cell types, therefore, are due not to different genes being present, but to **differential gene expression**, the expression of different genes by cells with the same genome.

The function of any cell, whether a single-celled eukaryote or a particular cell type in a multicellular organism, depends on the appropriate set of genes being expressed. The transcription factors of a cell must locate the right genes at the right time, a task on a par with finding a needle in a haystack. When gene expression proceeds abnormally, serious imbalances and diseases, including cancer, can arise.

Figure 18.6 summarizes the process of gene expression in a eukaryotic cell, highlighting key stages in the expression of a protein-coding gene. Each stage depicted in Figure 18.6

Figure 18.6 Stages in gene expression that can be regulated in eukaryotic cells. In this diagram, the colored boxes indicate the processes most often regulated; each color indicates the type of molecule that is affected (blue = DNA, red/orange = RNA, purple = protein). The nuclear envelope separating transcription from translation in eukaryotic cells offers an opportunity for post-transcriptional control in the form of RNA processing that is absent in prokaryotes. In addition, eukaryotes have a greater variety of control mechanisms operating before transcription and after translation. A miniature version of this figure accompanies several figures later in the chapter as an orientation diagram.



is a potential control point at which gene expression can be turned on or off, accelerated, or slowed down.

Fifty or so years ago, an understanding of the mechanisms that control gene expression in eukaryotes seemed almost hopelessly out of reach. Since then, new research methods, notably advances in DNA technology (see Chapter 19), have enabled molecular biologists to uncover many details of eukaryotic gene regulation. In all organisms, gene expression is commonly controlled at transcription; regulation at this stage often occurs in response to signals coming from outside the cell, such as hormones or other signaling molecules. For this reason, the term *gene expression* is often equated with transcription for both bacteria and eukaryotes. While this may often be the case for bacteria, the greater complexity of eukaryotic cell structure and function provides opportunities for regulating gene expression at many additional stages (see Figure 18.6). In the remainder of this section, we'll examine some of the important control points of eukaryotic gene expression more closely.

Regulation of Chromatin Structure

Recall that the DNA of eukaryotic cells is packaged with proteins in an elaborate complex known as chromatin, the basic unit of which is the nucleosome (see Figure 16.22). The structural organization of chromatin not only packs a cell's DNA into a compact form that fits inside the nucleus, but also helps regulate gene expression in several ways. The location of a gene's promoter, relative to both placement of nucleosomes and the sites where the DNA attaches to the chromosome scaffold, can affect whether the gene is transcribed. In addition, genes within heterochromatin, which is highly condensed, are usually not expressed. Lastly, certain chemical modifications to chromatin—both to the histone proteins of

the nucleosomes around which DNA is wrapped and to the nucleotides that make up that DNA—can influence chromatin structure and gene expression. Here we examine the effects of these modifications, which are catalyzed by specific enzymes.

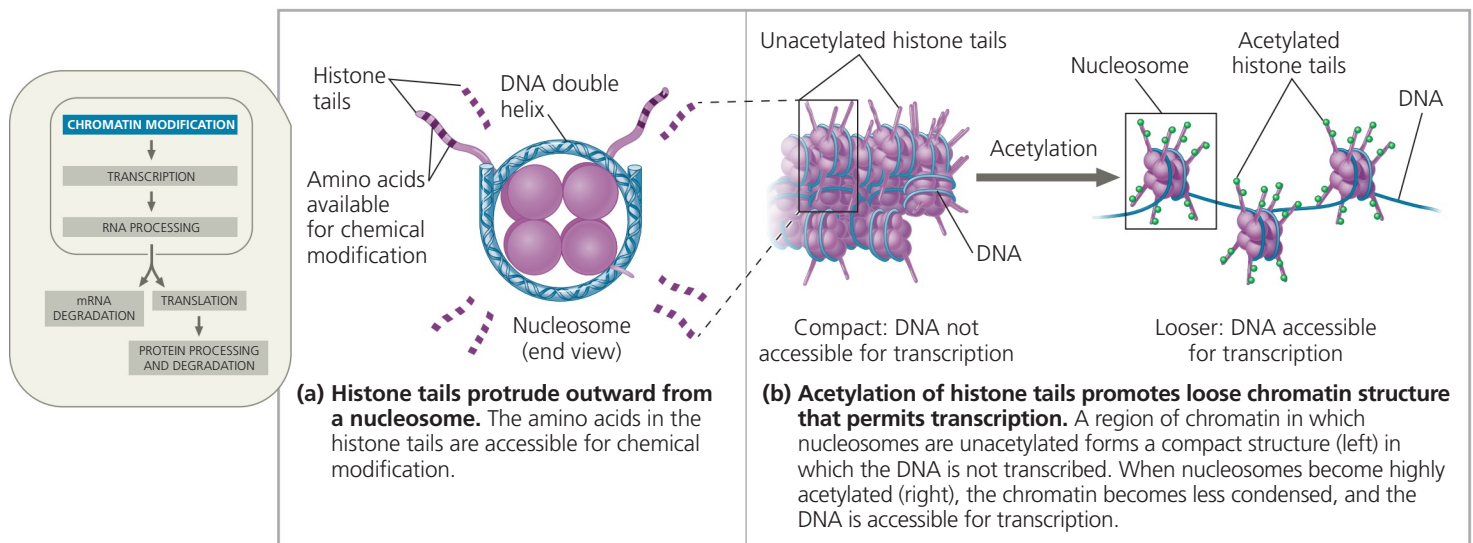
Histone Modifications and DNA Methylation

There is abundant evidence that chemical modifications to histones, found in all eukaryotic organisms, play a direct role in the regulation of gene transcription. The N-terminus of each histone protein in a nucleosome protrudes outward from the nucleosome (**Figure 18.7a**). These so-called *histone tails* are accessible to various modifying enzymes that catalyze the addition or removal of specific chemical groups, such as acetyl ($-\text{COCH}_3$), methyl, and phosphate groups (see Figure 4.9). Generally, **histone acetylation**—the addition of an acetyl group to an amino acid in a histone tail—appears to promote transcription by opening up the chromatin structure (**Figure 18.7b**), while the addition of methyl groups to histones can lead to the condensation of chromatin and reduced transcription. Often, the addition of a particular chemical group may create a new binding site for enzymes that further modify chromatin structure in various ways.

Rather than modifying histone proteins, a different set of enzymes can methylate the DNA itself on certain bases, usually cytosine. Such **DNA methylation** occurs in most plants, animals, and fungi. Long stretches of inactive DNA, such as that of inactivated mammalian X chromosomes (see Figure 15.8), are generally more methylated than regions of actively transcribed DNA (although there are exceptions). On a smaller scale, the DNA of individual genes is usually more heavily methylated in cells in which those genes are not expressed. Removal of the extra methyl groups can turn on some of these genes.

▼ Figure 18.7 A simple model of histone tails and the effect of histone acetylation.

In addition to acetylation, histones can undergo several other types of modifications, such as methylation or phosphorylation. These also help determine the chromatin configuration in a region, sometimes by establishing binding sites for chromatin-modifying enzymes.



Once methylated, genes usually stay that way through successive cell divisions in a given individual. At DNA sites where one strand is already methylated, enzymes methylate the correct daughter strand after each round of DNA replication. Methylation patterns are thus passed on to daughter cells, and cells forming specialized tissues keep a chemical record of what occurred during embryonic development. A methylation pattern maintained in this way also accounts for *genomic imprinting* in mammals, where methylation permanently regulates expression of either the maternal or paternal allele of particular genes at the start of development (see Figure 15.17).

Epigenetic Inheritance

The chromatin modifications that we just discussed do not change the DNA sequence, yet they still may be passed along to future generations of cells. Inheritance of traits transmitted by mechanisms not involving the nucleotide sequence itself is called **epigenetic inheritance**. Whereas mutations in the DNA are permanent changes, modifications to the chromatin can be reversed. For example, DNA methylation patterns are largely erased and reestablished during gamete formation.

Researchers are amassing more and more evidence for the importance of epigenetic information in the regulation of gene expression. Epigenetic variations might help explain why one identical twin acquires a genetically based disease, such as schizophrenia, but the other does not, despite their

identical genomes. Alterations in normal patterns of DNA methylation are seen in some cancers, where they are associated with inappropriate gene expression. Evidently, enzymes that modify chromatin structure are integral parts of the eukaryotic cell's machinery for regulating transcription.

Regulation of Transcription Initiation

Chromatin-modifying enzymes provide initial control of gene expression by making a region of DNA either more or less able to bind the transcription machinery. Once the chromatin of a gene is optimally modified for expression, the initiation of transcription is the next major step at which gene expression is regulated. As in bacteria, the regulation of transcription initiation in eukaryotes involves proteins that bind to DNA and either facilitate or inhibit binding of RNA polymerase. The process is more complicated in eukaryotes, however. Before looking at how eukaryotic cells control their transcription, let's review the structure of a eukaryotic gene.

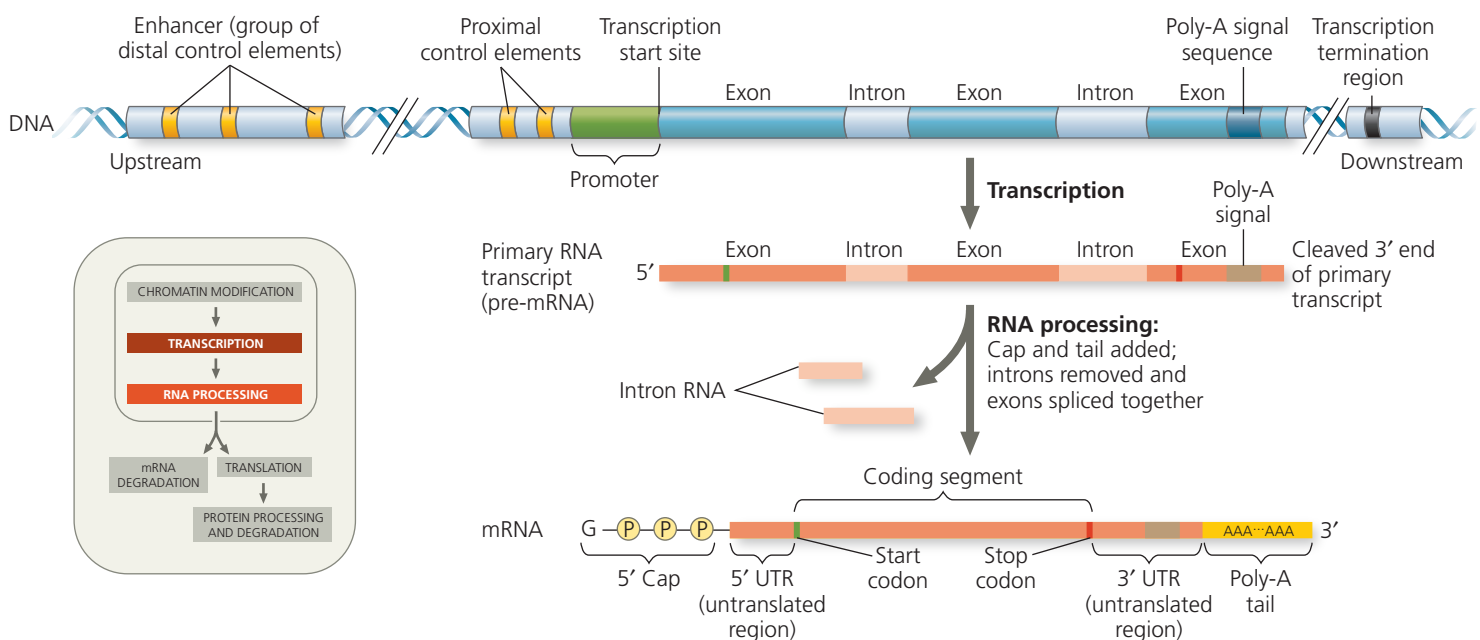
Organization of a Typical Eukaryotic Gene and Its Transcript

A eukaryotic gene and the DNA elements (segments) that control it are typically organized as shown in **Figure 18.8**, which extends what you learned about eukaryotic genes in Chapter 17. Recall that a cluster of proteins called a *transcription initiation complex* assembles on the promoter sequence

▼ **Figure 18.8 A eukaryotic gene and its transcript.** Each eukaryotic gene has a promoter—a DNA sequence where RNA polymerase binds and starts transcription, proceeding “downstream.” A number of control elements (gold) are involved in regulating the initiation of transcription; these are DNA sequences located near (proximal to) or far from

(distal to) the promoter. Distal control elements can be grouped together as enhancers, one of which is shown for this gene. At the other end of the gene, a polyadenylation (poly-A) signal sequence in the last exon of the gene is transcribed into an RNA sequence that signals where the transcript is cleaved and the poly-A tail added. Transcription may continue for hundreds

of nucleotides beyond the poly-A signal before terminating. RNA processing of the primary transcript into a functional mRNA involves three steps: addition of the 5' cap, addition of the poly-A tail, and splicing. In the cell, the 5' cap is added soon after transcription is initiated, and splicing occurs while transcription is still under way (see Figure 17.11).



at the “upstream” end of the gene (see Figure 17.9). One of these proteins, RNA polymerase II, then proceeds to transcribe the gene, synthesizing a primary RNA transcript (pre-mRNA). RNA processing includes enzymatic addition of a 5' cap and a poly-A tail, as well as splicing out of introns, to yield a mature mRNA. Associated with most eukaryotic genes are multiple **control elements**, segments of noncoding DNA that serve as binding sites for the proteins called transcription factors, which bind to the control elements and regulate transcription. Control elements on the DNA and the transcription factors that bind to them are critical to the precise regulation of gene expression seen in different cell types.

The Roles of General and Specific Transcription Factors

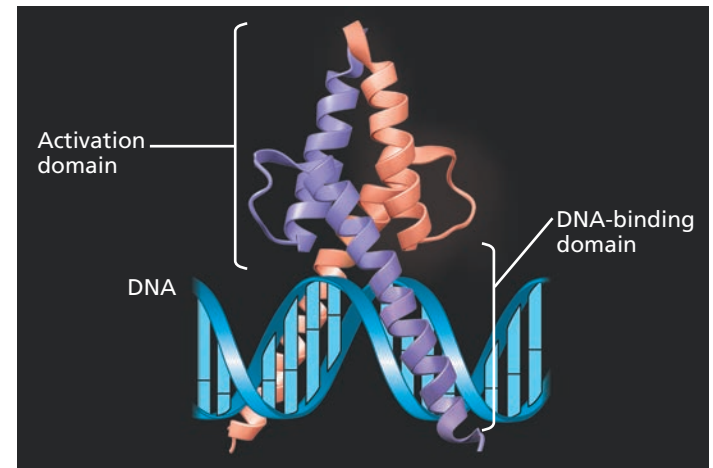
There are two types of transcription factors: General transcription factors act at the promoter of all genes, while some genes require specific transcription factors that bind to control elements that may be close to or farther away from the promoter.

General Transcription Factors at the Promoter To initiate transcription, eukaryotic RNA polymerase requires the assistance of transcription factors. Some transcription factors (such as those illustrated in Figure 17.9) are essential for the transcription of *all* protein-coding genes; therefore, they are often called *general transcription factors*. A few general transcription factors bind to a DNA sequence such as the TATA box within the promoter, but many bind to proteins, including other transcription factors and RNA polymerase II. Protein-protein interactions are crucial to the initiation of eukaryotic transcription. Only when the complete initiation complex has assembled can the polymerase begin to move along the DNA template strand, producing a complementary strand of RNA.

The interaction of general transcription factors and RNA polymerase II with a promoter usually leads to a low rate of initiation and production of few RNA transcripts from genes that are not expressed all the time, but instead are regulated. In eukaryotes, high levels of transcription of these particular genes at the appropriate time and place depend on the interaction of control elements with another set of proteins, which can be thought of as *specific transcription factors*.

Enhancers and Specific Transcription Factors As you can see in Figure 18.8, some control elements, named *proximal control elements*, are located close to the promoter. (Although some biologists consider proximal control elements part of the promoter, in this text we do not.) The more distant *distal control elements*, groupings of which are called **enhancers**, may be thousands of nucleotides upstream or downstream of a gene or even within an intron. A given gene may have multiple enhancers, each active at a different time or in a different cell type or location in the organism. Each enhancer, however, is generally associated with only that gene and no other.

▼ **Figure 18.9 The structure of MyoD, a transcriptional activator.** The MyoD protein is made up of two polypeptide subunits (purple and salmon) with extensive regions of α helix. Each subunit has one DNA-binding domain (lower half) and one activation domain (upper half). The latter includes binding sites for the other subunit and for other proteins. MyoD is involved in muscle development in vertebrate embryos (see Concept 18.4).



VISUAL SKILLS ► Describe how the two functional domains of the MyoD protein relate to the two polypeptide subunits.

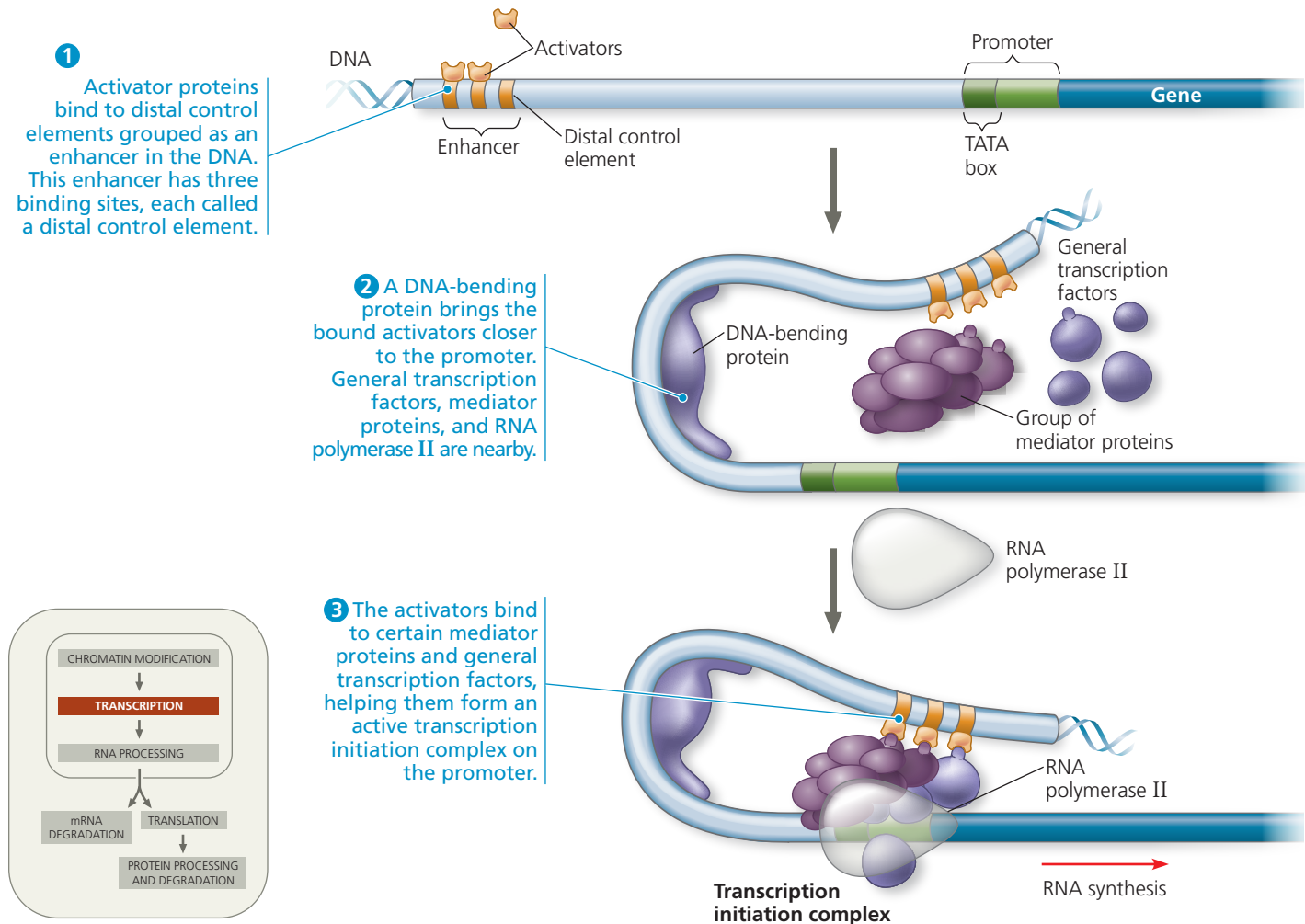
In eukaryotes, the rate of gene expression can be strongly increased or decreased by the binding of specific transcription factors, either activators or repressors, to the control elements of enhancers. Hundreds of transcription activators have been discovered in eukaryotes; the structure of one example is shown in **Figure 18.9**. Researchers have identified two types of structural domains that are commonly found in a large number of activator proteins: a *DNA-binding domain*—a part of the protein’s three-dimensional structure that binds to DNA—and one or more *activation domains*. Activation domains bind other regulatory proteins or components of the transcription machinery, facilitating a series of protein-protein interactions that result in enhanced transcription of a given gene.

How can binding of activators to an enhancer located far from the promoter influence transcription? One study shows that the proteins regulating a mouse globin gene contact both the gene’s promoter and an enhancer located about 50,000 nucleotides upstream. This and many other studies support the currently accepted model, in which protein-mediated bending of the DNA is thought to bring the bound activators into contact with a group of *mediator proteins*, which in turn interact with general transcription factors at the promoter (**Figure 18.10**). These protein-protein interactions help assemble and position the initiation complex on the promoter, and allow the promoter and enhancer to come together in a very specific fashion, in spite of what is often a large number of nucleotide pairs between them. In the **Scientific Skills Exercise**, you can work with data from an experiment that identified the control elements in an enhancer of a particular human gene.

▼ **Figure 18.10 A model for the action of enhancers and transcription activators.** Bending of the DNA by a protein enables enhancers to influence a promoter hundreds or even thousands of nucleotides away. Specific transcription factors called

activators bind to the enhancer DNA sequences and then to a group of mediator proteins. These in turn bind to general transcription factors and then RNA polymerase II, thus assembling the transcription initiation complex. These protein-protein interactions lead to

correct positioning of the complex on the promoter and the initiation of RNA synthesis. Only one enhancer (with three gold control elements) is shown here, but a gene may have several enhancers that act at different times or in different cell types.



Animation: Regulation of Transcription by Transcriptional Activators and Enhancers

Specific transcription factors that function as repressors can inhibit gene expression in several different ways. Some repressors bind directly to control element DNA (in enhancers or elsewhere), blocking activator binding. Other repressors interfere with the activator itself so it can't bind the DNA.

In addition to influencing transcription directly, some activators and repressors act indirectly by affecting chromatin structure. Studies using yeast and mammalian cells show that some activators recruit proteins that acetylate histones near the promoters of specific genes, thus promoting transcription (see Figure 18.7). Similarly, some repressors recruit proteins that remove acetyl groups from histones, leading to reduced transcription, a phenomenon referred to as

silencing. Indeed, recruitment of chromatin-modifying proteins seems to be the most common mechanism of repression in eukaryotic cells.

Combinatorial Control of Gene Activation In eukaryotes, the precise control of transcription depends largely on the binding of activators to DNA control elements. Considering the great number of genes that must be regulated in a typical animal or plant cell, the number of completely different nucleotide sequences found in control elements is surprisingly small. A dozen or so short nucleotide sequences appear again and again in the control elements for different genes. On average, each enhancer is composed of about ten control elements, each of which can

SCIENTIFIC SKILLS EXERCISE

Analyzing DNA Deletion Experiments



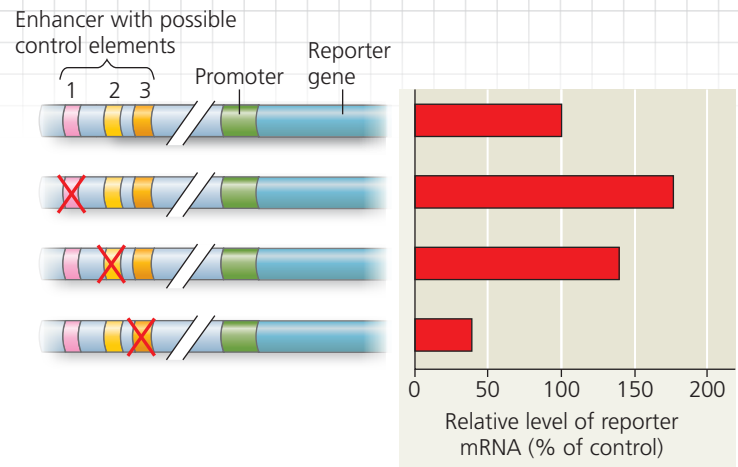
What Control Elements Regulate Expression of the *mPGES-1* Gene?

The promoter of a gene includes the DNA immediately upstream of the transcription start site, but the control elements that regulate the level of transcription of the gene (grouped in an enhancer) may be thousands of base pairs upstream of the promoter. Because the distance and spacing of control elements make them difficult to identify, scientists begin by deleting pos-

sible control elements and measuring the effect on gene expression. In this exercise, you will analyze data obtained from DNA deletion experiments that tested possible control elements for the human gene *mPGES-1*. This gene codes for an enzyme that synthesizes a type of prostaglandin, a chemical made during tissue inflammation.

How the Experiment Was Done The researchers hypothesized that there were three possible control elements in an enhancer region located 8–9 kilobases upstream of the *mPGES-1* gene. Control elements regulate whatever gene is in the appropriate downstream location. Thus, to test the activity of the possible elements, researchers first synthesized molecules of DNA (“constructs”) that had the intact enhancer region upstream of a “reporter gene,” a gene whose mRNA product could be easily measured experimentally. Next, they made three more DNA constructs, with one of the three proposed control elements deleted in each (see the left side of the figure). The researchers then introduced each DNA construct into a separate human cell culture, where the cells took up the DNA constructs. After 48 hours, the amount of reporter gene mRNA made by the cells was measured. Comparing these amounts allowed researchers to determine if any of the deletions had an effect on expression of the reporter gene, mimicking the effect of deletions on *mPGES-1* gene expression. (The *mPGES-1* gene itself couldn’t be used to measure expression levels because the cells express their own *mPGES-1* gene, and the mRNA from that gene would confuse the results.)

Data from the Experiment The diagrams on the left side of the figure show the intact DNA sequence (top) and the three experimental DNA constructs. A red X is located on the possible control element (1, 2, or 3) that was deleted in each experimental DNA construct. The area between the slashes represents the approximately 8 kilobases of DNA located between the promoter and the enhancer region. The horizontal bar graph on the right shows the amount of



Data from J. N. Walters et al., Regulation of human microsomal prostaglandin E synthase-1 by IL-1b requires a distal enhancer element with a unique role for C/EBPb, *Biochemical Journal* 443:561–571 (2012).

reporter gene mRNA that was present in each cell culture after 48 hours relative to the amount that was in the culture containing the intact enhancer region (top bar = 100%).

INTERPRET THE DATA

- (a) What is the independent variable in the graph? (b) What is the dependent variable? (c) What was the control treatment in this experiment? Label it on the diagram.
- Do the data suggest that any of these possible control elements are actual control elements? Explain.
- (a) Did deletion of any of the possible control elements cause a *reduction* in reporter gene expression? If so, which one(s), and how can you tell? (b) If loss of a control element causes a reduction in gene expression, what must be the normal role of that control element? Provide a biological explanation for how the loss of such a control element could lead to a reduction in gene expression.
- (a) Did deletion of any of the possible control elements cause an *increase* in reporter gene expression relative to the control? If so, which one(s), and how can you tell? (b) If loss of a control element causes an increase in gene expression, what must be the normal role of that control element? Propose a biological explanation for how the loss of such a control element could lead to an increase in gene expression.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

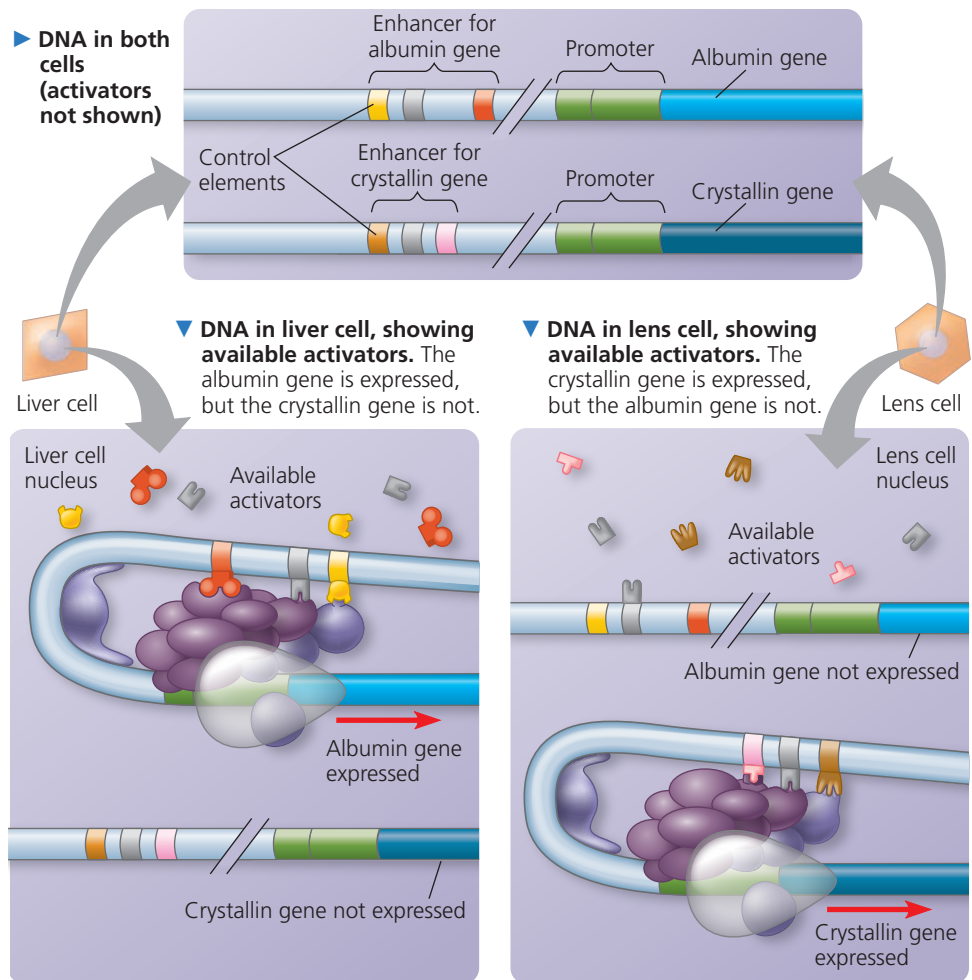
bind only one or two specific transcription factors. It is the particular *combination* of control elements in an enhancer associated with a gene, rather than the presence of a single unique control element, that is important in regulating transcription of the gene.

Even with only a dozen control element sequences available, a very large number of combinations is possible. Each combination of control elements is able to activate transcription only when the appropriate activator proteins are present,

which may occur at a precise time during development or in a particular cell type. **Figure 18.11** illustrates how the use of different combinations of just a few control elements can allow differential regulation of transcription in two representative cell types—liver cells and lens cells. This can occur because each cell type contains a different group of activator proteins. How cell types come to differ during this process, even though they all arise from one cell (the fertilized egg), will be explored in Concept 18.4.

► **Figure 18.11 Cell type-specific transcription.** Both liver cells and lens cells have the genes for making the proteins albumin and crystallin, but only liver cells make albumin (a blood protein) and only lens cells make crystallin (the main protein of the lens of the eye). The specific transcription factors made in a cell determine which genes are expressed. In this example, the genes for albumin and crystallin are shown at the top, each with an enhancer made up of three different control elements. Although the enhancers for the two genes both have a gray control element, each enhancer has a unique *combination* of elements. All the activator proteins required for high-level expression of the albumin gene are present in liver cells only (left), whereas the activators needed for expression of the crystallin gene are present in lens cells only (right). For simplicity, we consider only the role of specific transcription factors that are activators here, although repressors may also influence transcription in certain cell types.

VISUAL SKILLS ► Describe the enhancer for the albumin gene in each type of cell. How would the nucleotide sequence of this enhancer in the liver cell compare with that in the lens cell?



Coordinately Controlled Genes in Eukaryotes

How does the eukaryotic cell deal with a group of genes of related function that need to be turned on or off at the same time? Earlier in this chapter, you learned that in bacteria, such *coordinately controlled* genes are often clustered into an operon, which is regulated by a single promoter and transcribed into a single mRNA molecule. Thus, the genes are expressed together, and the encoded proteins are produced concurrently. With a few exceptions, operons that work in this way have *not* been found in eukaryotic cells.

Eukaryotic genes that are co-expressed, such as genes coding for the enzymes of a metabolic pathway, are typically scattered over different chromosomes. Here, coordinate gene expression depends on every gene of a dispersed group having a specific combination of control elements. Activator proteins in the nucleus that recognize the control elements bind to them, promoting simultaneous transcription of the genes, no matter where they are in the genome.

Coordinate control of dispersed genes in a eukaryotic cell often occurs in response to chemical signals from outside the cell. A steroid hormone, for example, enters a cell and binds to a specific intracellular receptor protein, forming a hormone-receptor complex that serves as a transcription

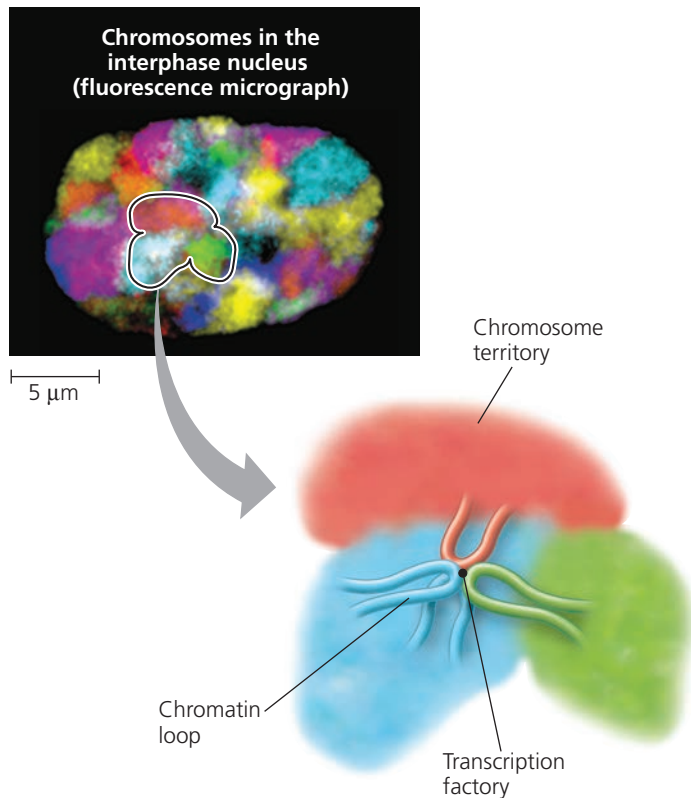
activator (see Figure 9.9). Every gene whose transcription is stimulated by a given steroid hormone, regardless of its chromosomal location, has a control element recognized by that hormone-receptor complex. This is how estrogen activates a group of genes that stimulate cell division in uterine cells, preparing the uterus for pregnancy.

Many signaling molecules, such as nonsteroid hormones and growth factors, bind to receptors on a cell's surface and never actually enter the cell. Such molecules can control gene expression indirectly by triggering signal transduction pathways that activate particular transcription factors (see Figure 9.15). Coordinate regulation in such pathways is the same as for steroid hormones: Genes with the same sets of control elements are activated by the same chemical signals. Because this system for coordinating gene regulation is so widespread, biologists think that it probably arose early in evolutionary history.

Nuclear Architecture and Gene Expression

You saw in Figure 16.23b that each chromosome in the interphase nucleus occupies a distinct territory. The chromosomes are not completely isolated, however. Recently, *chromosome conformation capture* (3C) techniques have been developed that allow researchers to cross-link and identify regions of

▼ **Figure 18.12 Chromosomal interactions in the interphase nucleus.** Although each chromosome has its own territory (see Figure 16.23b), loops of chromatin may extend into other sites in the nucleus. Some of these sites are transcription factories that are occupied by multiple chromatin loops from the same chromosome (blue loops) or other chromosomes (red and green loops).



chromosomes that associate with each other during interphase. These studies reveal that loops of chromatin extend from individual chromosomal territories into specific sites in the nucleus (**Figure 18.12**). Different loops from the same chromosome and loops from other chromosomes may congregate in such sites, some of which are rich in RNA polymerases and other transcription-associated proteins. Like a recreation center that draws members from many different neighborhoods, these so-called *transcription factories* are thought to be areas specialized for a common function.

The old view that the nuclear contents are like a bowl of amorphous chromosomal spaghetti has given way to a new model of a nucleus with a defined architecture and regulated movements of chromatin. Several lines of evidence suggest that genes that are not being expressed are located in the outer edges of the nucleus, while those that are being expressed are found in its interior region. Relocation of particular genes from their chromosomal territories to transcription factories in the interior may be part of the process of readying genes for transcription. How long an individual transcription factory may last has not yet been established. In 2014, the National Institutes of Health announced funding for a new “4D Nucleome” program, which aims to investigate the many fascinating questions addressed by this exciting area of current research.

Mechanisms of Post-Transcriptional Regulation

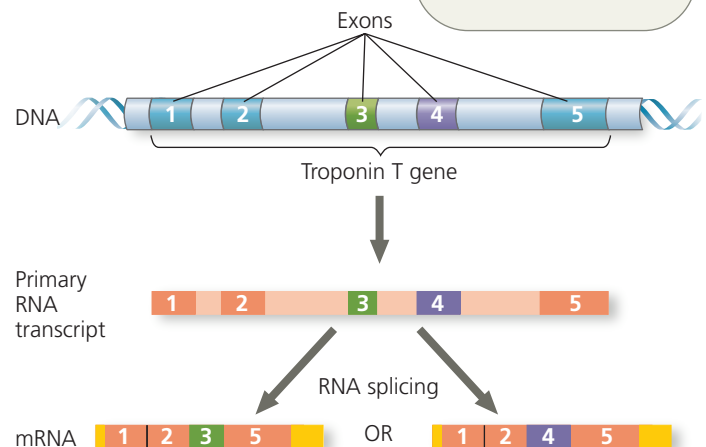
Transcription alone does not constitute gene expression. The expression of a protein-coding gene is ultimately measured by the amount of functional protein a cell makes, and much happens between the synthesis of the RNA transcript and the activity of the protein in the cell. Many regulatory mechanisms operate at the various stages after transcription (see Figure 18.6). These mechanisms allow a cell to rapidly fine-tune gene expression in response to environmental changes without altering its transcription patterns. Here we discuss how cells can regulate gene expression once a gene has been transcribed.

RNA Processing

RNA processing in the nucleus and the export of mature RNA to the cytoplasm provide opportunities for regulating gene expression not available in prokaryotes. One example of regulation at the RNA-processing level is **alternative RNA splicing**, in which different mRNA molecules are produced from the same primary transcript, depending on which RNA segments are treated as exons and which as introns. Regulatory proteins specific to a cell type control intron-exon choices by binding to regulatory sequences within the primary transcript.

A simple example of alternative RNA splicing is shown in **Figure 18.13** for the troponin T gene, which encodes two different (though related) proteins. Other genes code for many more possible products. For instance, researchers have found a *Drosophila* gene with enough alternatively spliced exons to

▼ **Figure 18.13 Alternative RNA splicing of the troponin T gene.** The primary transcript of this gene can be spliced in more than one way, generating different mRNA molecules. Notice that one mRNA molecule has ended up with exon 3 (green) and the other with exon 4 (purple). These two mRNAs are translated into different but related muscle proteins.



generate about 19,000 membrane proteins that have different extracellular domains. At least 17,500 (94%) of the alternative mRNAs are actually synthesized. Each developing nerve cell in the fly appears to synthesize a different form of the protein, which acts as unique identifier on the cell surface and helps prevent excessive overlap of nerve cells during development of the nervous system.

It is clear that alternative RNA splicing can significantly expand the repertoire of a eukaryotic genome. In fact, alternative splicing was proposed as one explanation for the surprisingly low number of human genes counted when the human genome was sequenced. The number of human genes was found to be similar to that of a soil worm (nematode), a mustard plant, or a sea anemone. This discovery prompted questions about what, if not the number of genes, accounts for the more complex morphology (external form) of humans. It turns out that more than 90% of human protein-coding genes probably undergo alternative splicing. Thus, the extent of alternative splicing greatly multiplies the number of possible human proteins, which may be better correlated with complexity of form.

Initiation of Translation and mRNA Degradation

Translation is another opportunity for regulating gene expression; it occurs most commonly at the initiation stage (see Figure 17.19). For some mRNAs, the initiation of translation can be blocked by regulatory proteins that bind to specific sequences or structures within the untranslated region (UTR) at the 5' or 3' end, preventing the attachment of ribosomes. (Recall from Concept 17.3 that both the 5' cap and the poly-A tail of an mRNA molecule are important for ribosome binding.)

Alternatively, translation of *all* the mRNAs in a cell may be regulated simultaneously. In a eukaryotic cell, such “global” control usually involves the activation or inactivation of one or more of the protein factors required to initiate translation. This mechanism plays a role in starting translation of mRNAs that are stored in eggs. Just after fertilization, translation is triggered by the sudden activation of translation initiation factors. The response is a burst of synthesis of the proteins encoded by the stored mRNAs. Some plants and algae store mRNAs during periods of darkness; light then triggers the reactivation of the translational apparatus.

The life span of mRNA molecules in the cytoplasm is important in determining the pattern of protein synthesis in a cell. Bacterial mRNA molecules typically are degraded by enzymes within a few minutes of their synthesis. This short life span of mRNAs is one reason bacteria can change their patterns of protein synthesis so quickly in response to environmental changes. In contrast, mRNAs in multicellular eukaryotes typically survive for hours, days, or even weeks. For instance, the mRNAs for the hemoglobin polypeptides (α -globin and β -globin) in developing red blood cells are unusually stable, and these long-lived mRNAs are translated repeatedly in red blood cells.

Nucleotide sequences that affect how long an mRNA remains intact are often found in the untranslated region at the 3' end of the molecule (see Figure 18.8). In one experiment, researchers transferred such a sequence from the short-lived mRNA for a growth factor to the 3' end of a normally stable globin mRNA. The globin mRNA was quickly degraded.

During the past few years, other mechanisms that degrade or block expression of mRNA molecules have come to light. They involve a group of newly discovered RNA molecules that regulate gene expression at several levels, as we'll discuss shortly.

Protein Processing and Degradation

The final opportunities for controlling gene expression occur after translation. Often, eukaryotic polypeptides must be processed to yield functional protein molecules. For instance, cleavage of the initial insulin polypeptide (pro-insulin) forms the active hormone. In addition, many proteins undergo chemical modifications that make them functional. Regulatory proteins are commonly activated or inactivated by the reversible addition of phosphate groups (see Figure 9.10), and proteins destined for the surface of animal cells acquire sugars (see Figure 7.12). Cell-surface proteins and many others must also be transported to target destinations in the cell in order to function (see Figure 17.22). Regulation might occur at any of the steps involved in modifying or transporting a protein.

Finally, the length of time each protein functions in the cell is strictly regulated by selective degradation. Many proteins, such as the cyclins involved in regulating the cell cycle, must be relatively short-lived if the cell is to function appropriately (see Figure 12.16). To mark a protein for destruction, the cell commonly attaches molecules of a small protein called ubiquitin to the protein. Giant protein complexes called proteasomes then recognize the ubiquitin-tagged proteins and degrade them.



Animation: Post-Transcriptional Control Mechanisms

CONCEPT CHECK 18.2

1. In general, what are the effects of histone acetylation and DNA methylation on gene expression?
2. **MAKE CONNECTIONS** > Speculate about whether the same enzyme could methylate both a histone and a DNA base. (See Concept 5.4.)
3. Compare the roles of general and specific transcription factors in regulating gene expression.
4. Once mRNA encoding a particular protein reaches the cytoplasm, what are four mechanisms that can regulate the amount of the protein that is active in the cell?
5. **WHAT IF?** > Suppose you compared the nucleotide sequences of the distal control elements in the enhancers of three genes that are expressed only in muscle cells. What would you expect to find? Why?

For suggested answers, see Appendix A.

CONCEPT 18.3

Noncoding RNAs play multiple roles in controlling gene expression

Genome sequencing has revealed that protein-coding DNA accounts for only 1.5% of the human genome and a similarly small percentage of the genomes of many other multicellular eukaryotes. A very small fraction of the non-protein-coding DNA consists of genes for RNAs such as ribosomal RNA and transfer RNA. Until recently, scientists assumed that most of the remaining DNA was not transcribed, thinking that since it didn't specify proteins or the few known types of RNA, such DNA didn't contain meaningful genetic information—in fact, it was called “junk DNA.” However, a flood of recent data has contradicted this idea. For example, a massive study of the entire human genome showed that roughly 75% of the genome is transcribed at some point in any given cell. Introns account for only a fraction of this transcribed, nontranslated RNA. These and other results suggest that a significant amount of the genome may be transcribed into non-protein-coding RNAs—also called *noncoding RNAs*, or *ncRNAs*—including a variety of small RNAs. Researchers are uncovering more evidence of the biological roles of these ncRNAs every day.

Biologists are excited about these discoveries, which have revealed a large and diverse population of RNA molecules in the cell that play crucial roles in regulating gene expression—but have gone largely unnoticed until fairly recently. Our long-standing view that because mRNAs code for proteins, they are the most important RNAs functioning in the cell demands revision. This represents a major shift in the thinking of biologists, one that you are witnessing as students entering this field of study.

Effects on mRNAs by MicroRNAs and Small Interfering RNAs

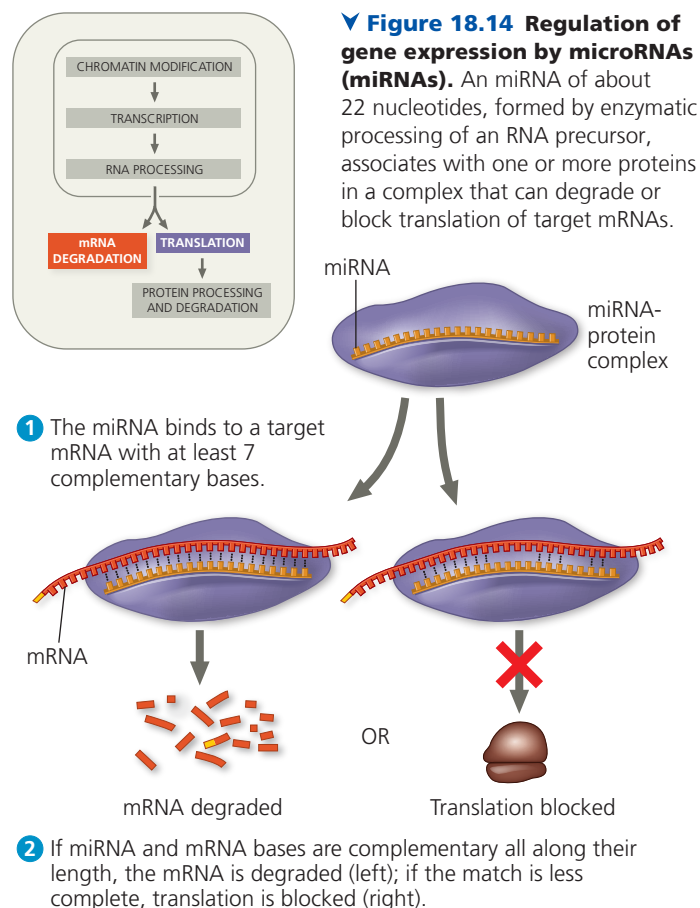
Regulation by both small and large ncRNAs occurs at several points in the pathway of gene expression, including mRNA translation and chromatin modification. We'll examine two types of small ncRNAs, the importance of which was acknowledged when their discovery was the focus of the 2006 Nobel Prize in Physiology or Medicine, which was awarded for work completed only eight years earlier.

Since 1993, a number of research studies have uncovered **microRNAs (miRNAs)**—small, single-stranded RNA molecules capable of binding to complementary sequences in mRNA molecules. A longer RNA precursor is processed by cellular enzymes into an miRNA, a single-stranded RNA of about 22 nucleotides that forms a complex with one or more proteins (**Figure 18.14**). The miRNA allows the complex to bind to any mRNA molecule with at least 7 or 8 nucleotides of complementary sequence. The miRNA-protein complex

then degrades the target mRNA or, less often, simply blocks its translation. There are approximately 1,500 genes for miRNAs in the human genome, and biologists estimate that expression of at least one-half of all human genes may be regulated by miRNAs, a remarkable figure given that the existence of miRNAs was unknown 25 years ago.

Another class of small RNAs, similar in size and function to miRNAs, is called **small interfering RNAs (siRNAs)**. Both miRNAs and siRNAs can associate with the same proteins, producing similar results. In fact, if siRNA precursor RNA molecules are injected into a cell, the cell's machinery can process them into siRNAs that turn off expression of genes with related sequences, similarly to how miRNAs function. The distinction between miRNAs and siRNAs is based on subtle differences in the structure of their precursors, which in both cases are RNA molecules that are mostly double-stranded. The blocking of gene expression by siRNAs is referred to as **RNA interference (RNAi)**, and it is used in the laboratory as a means of disabling specific genes to investigate their function.

How did the RNAi pathway evolve? As you will learn in Concept 26.2, some viruses have double-stranded RNA genomes. Given that the cellular RNAi pathway can process double-stranded RNAs into homing devices that lead to destruction of related RNAs, some scientists think that this pathway may have evolved as a natural defense against



chromosome to be inactivated—bind back to and coat that chromosome, and this binding leads to condensation of the entire chromosome into heterochromatin.

The cases described above involve chromatin remodeling in large regions of the chromosome. Because chromatin structure affects transcription and thus gene expression, RNA-based regulation of chromatin structure is sure to play an important role in gene regulation. Additionally, some experimental evidence supports the idea of an alternate role for lncRNAs in which they can act as a scaffold, bringing together DNA, proteins, and other RNAs into complexes. These associations may act either to condense chromatin or, in some cases, to help bring the enhancer of a gene together with mediator proteins and the gene's promoter, activating gene expression in a more direct fashion.

The Evolutionary Significance of Small ncRNAs

EVOLUTION Small ncRNAs can regulate gene expression at multiple steps and in many ways. While this section has focused on ncRNAs in eukaryotes, small ncRNAs are also used by bacteria as a defense system, called the CRISPR-Cas9 system, against viruses that infect them. (You'll learn more about this in Concept 26.2.) The use of ncRNAs thus evolved long ago, but we don't yet know how bacterial ncRNAs are related to those of eukaryotes.

What about the evolutionary significance of small eukaryotic ncRNAs? In general, extra levels of gene regulation might allow evolution of a higher degree of complexity of form. The versatility of miRNA regulation has therefore led some biologists to hypothesize that an increase in the number of different miRNAs specified by the genome of a given species has allowed morphological complexity to increase over evolutionary time. While this hypothesis is still being evaluated, it is logical to expand the discussion to include all small ncRNAs. Exciting new techniques for rapidly sequencing genomes have allowed biologists to begin asking how many genes for ncRNAs are present in the genome of any given species. A survey of different species supports the notion that siRNAs evolved first, followed by miRNAs and later piRNAs, which are found only in animals. And while there are hundreds of types of miRNAs, there appear to be 60,000 or so types of piRNAs, allowing the potential for very sophisticated gene regulation by piRNAs.

Given the extensive functions of ncRNAs, it is not surprising that many of the ncRNAs characterized thus far play important roles in embryonic development—the topic we turn to in the next section. Embryonic development is perhaps the ultimate example of precisely regulated gene expression.

CONCEPT CHECK 18.3

1. Compare miRNAs and siRNAs, including their functions.
2. **WHAT IF? >** Suppose the mRNA being degraded in Figure 18.14 coded for a protein that promotes cell division in a multicellular organism. What would happen if a mutation disabled the gene for the miRNA that triggers this degradation?
3. **MAKE CONNECTIONS >** Inactivation of one of the X chromosomes in female mammals involves lncRNA called *XIST* RNA, mentioned in this section and in Concept 15.2. Describe transcription and binding of *XIST* RNA, then suggest a model for how it initiates Barr body formation.

For suggested answers, see Appendix A.

CONCEPT 18.4

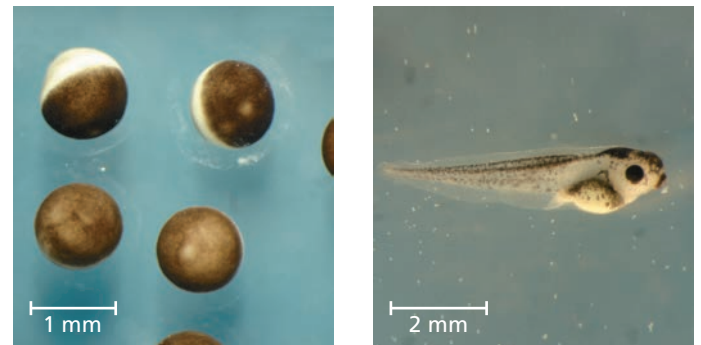
A program of differential gene expression leads to the different cell types in a multicellular organism

In the embryonic development of multicellular organisms, a fertilized egg (a zygote) gives rise to cells of many different types, each with a different structure and corresponding function. Typically, cells are organized into tissues, tissues into organs, organs into organ systems, and organ systems into the whole organism. Thus, any developmental program must produce cells of different types that form higher-level structures arranged in a particular way in three dimensions. The processes that occur during development in plants and animals are detailed in Chapters 35 and 46, respectively. In this chapter, we focus on the program of regulation of gene expression that orchestrates development, using a few animal species as examples.

A Genetic Program for Embryonic Development

The photos in **Figure 18.16** illustrate the dramatic difference between a frog zygote (fertilized egg) and the tadpole it

✓ Figure 18.16 From fertilized egg to animal: What a difference four days makes. It takes just four days for cell division, differentiation, and morphogenesis to transform each of the fertilized frog eggs shown in (a) into a tadpole like the one in (b).



(a) Fertilized eggs of a frog

(b) Newly hatched tadpole

becomes. This remarkable transformation results from three interrelated processes: cell division, cell differentiation, and morphogenesis. Through a succession of mitotic cell divisions, the zygote gives rise to a large number of cells. Cell division alone, however, would merely produce a great ball of identical cells, nothing like a tadpole. During embryonic development, cells not only increase in number, but also undergo cell **differentiation**, the process by which cells become specialized in structure and function. Moreover, the different kinds of cells are not randomly distributed but are organized into tissues and organs in a particular three-dimensional arrangement. The physical processes that give an organism its shape constitute **morphogenesis**, the development of the form of an organism and its structures.

All three processes are rooted in cellular behavior. Even morphogenesis, the shaping of the organism, can be traced back to changes in the shape, motility, and other characteristics of the cells that make up various regions of the embryo. As you have seen, the activities of a cell depend on the genes it expresses and the proteins it produces. Almost all cells in an organism have the same genome; therefore, differential gene expression results from the genes being regulated differently in each cell type.

In Figure 18.11, you saw a simplified view of how differential gene expression occurs in two cell types, a liver cell and a lens cell. Each of these fully differentiated cells has a particular mix of specific activators that turn on the collection of genes whose products are required in the cell. The fact that both cells arose through a series of mitoses from a common fertilized egg inevitably leads to a question: How do different sets of activators come to be present in the two cells?

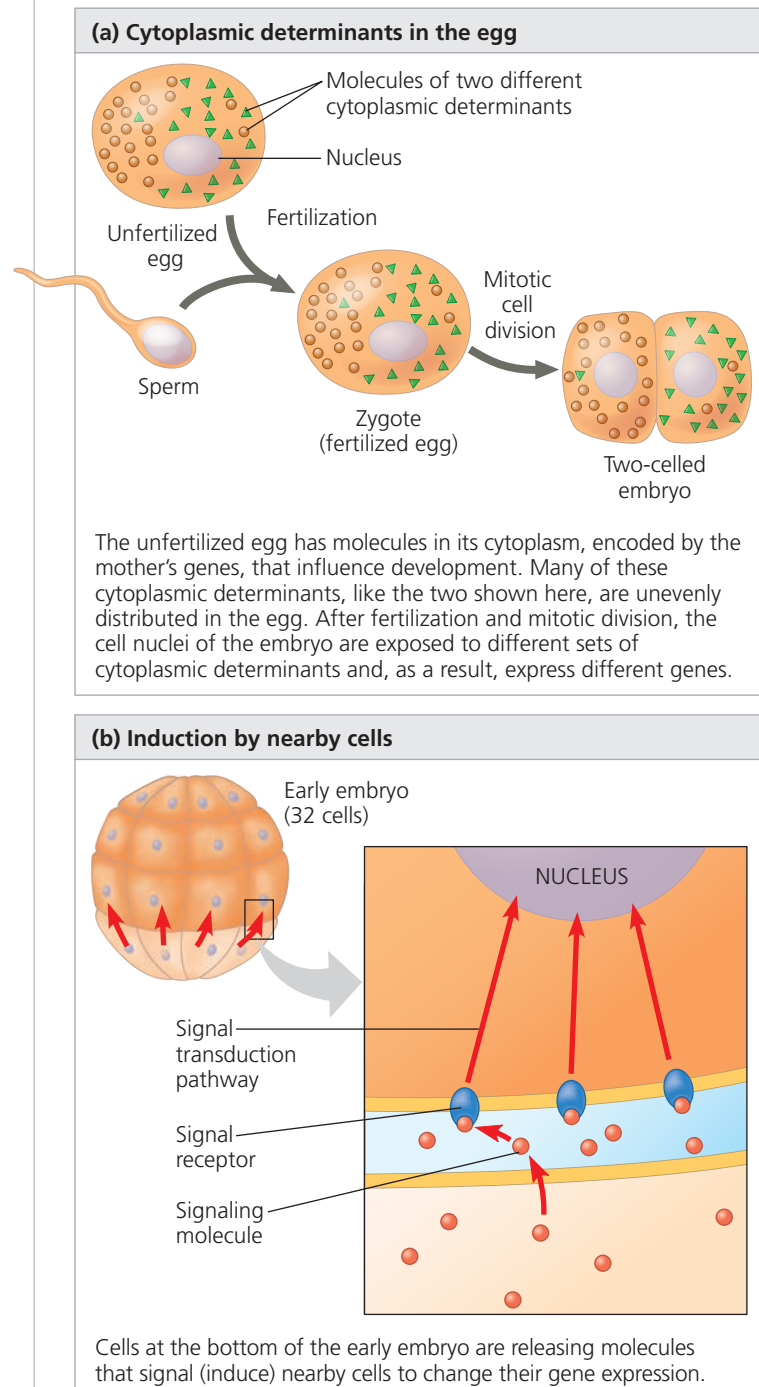
It turns out that materials placed into the egg by maternal cells set up a sequential program of gene regulation that is carried out as embryonic cells divide, and this program coordinates cell differentiation during embryonic development. To understand how this works, we will consider two basic developmental processes. First, we'll explore how cells that arise from early embryonic mitoses develop the differences that start each cell along its own differentiation pathway. Second, we'll see how cellular differentiation leads to one particular cell type, using muscle development as an example.

Cytoplasmic Determinants and Inductive Signals

What generates the first differences among cells in an early embryo? And what controls the differentiation of all the various cell types as development proceeds? By this point in the chapter, you can probably deduce the answer: The specific genes expressed in any particular cell of a developing organism determine its path. Two sources of information, used to varying extents in different species, “tell” a cell which genes to express at any given time during embryonic development.

One important source of information early in development is the egg's cytoplasm, which contains both RNA and proteins encoded by the mother's DNA. The cytoplasm of an unfertilized egg is not homogeneous. Messenger RNA, proteins, other substances, and organelles are distributed unevenly in the unfertilized egg, and this unevenness has a profound impact on the development of the future embryo in many species. Maternal substances in the egg that influence the course of early development are called **cytoplasmic determinants** (Figure 18.17a). After fertilization, early mitotic divisions distribute the zygote's

▼ **Figure 18.17 Sources of developmental information for the early embryo.**



cytoplasm into separate cells. The nuclei of these cells may thus be exposed to different cytoplasmic determinants, depending on which portions of the zygotic cytoplasm a cell received. The combination of cytoplasmic determinants in a cell helps determine its developmental fate by regulating expression of the cell's genes during the course of cell differentiation.

The other major source of developmental information, which becomes increasingly important as the number of embryonic cells increases, is the environment around a particular cell. Most influential are the signals conveyed to an embryonic cell from other embryonic cells in the vicinity, including contact with cell-surface molecules on neighboring cells and the binding of growth factors secreted by neighboring cells (see Concept 9.1). Such signals cause changes in the target cells, a process called **induction** (Figure 18.17b). The molecules passing along these signals within the target cell are cell-surface receptors and other signaling pathway proteins. In general, the signaling molecules send a cell down a specific developmental path by causing changes in its gene expression that eventually result in observable cellular changes. Thus, interactions between embryonic cells help induce differentiation into the many specialized cell types making up a new organism.

Sequential Regulation of Gene Expression During Cellular Differentiation

The earliest changes that set a cell on its path to specialization are subtle ones, showing up only at the molecular level. Before biologists knew much about the molecular changes occurring in embryos, they coined the term **determination** to refer to the point at which an embryonic cell is irreversibly committed to becoming a particular cell type. Once it has undergone determination, an embryonic cell can be experimentally placed in another location in the embryo and it will still differentiate into the cell type that is its normal fate. Differentiation, then, is the process by which a cell attains its determined fate. As the tissues and organs of an embryo develop and their cells differentiate, the cells become more noticeably different in structure and function.

Today we understand determination in terms of molecular changes. The outcome of determination, observable cell differentiation, is marked by the expression of genes for *tissue-specific proteins*. These proteins are found only in a specific cell type and give the cell its characteristic structure and function. The first evidence of differentiation is the appearance of mRNAs for these proteins. Eventually, differentiation is observable with a microscope as changes in cellular structure. On the molecular level, different sets of genes are sequentially expressed in a regulated manner as new cells arise from division of their precursors. A number of the steps in gene expression may be regulated during differentiation, transcription being the most common. In the fully differentiated cell, transcription remains the principal regulatory point for maintaining appropriate gene expression.

Differentiated cells are specialists at making tissue-specific proteins. For example, as a result of transcriptional regulation, liver cells specialize in making albumin, and lens cells specialize in making crystallin (see Figure 18.11). Skeletal muscle cells in vertebrates are another instructive example. Each of these cells is a long fiber containing many nuclei within a single plasma membrane. Skeletal muscle cells have high concentrations of muscle-specific versions of the contractile proteins myosin and actin, as well as membrane receptor proteins that detect signals from nerve cells.

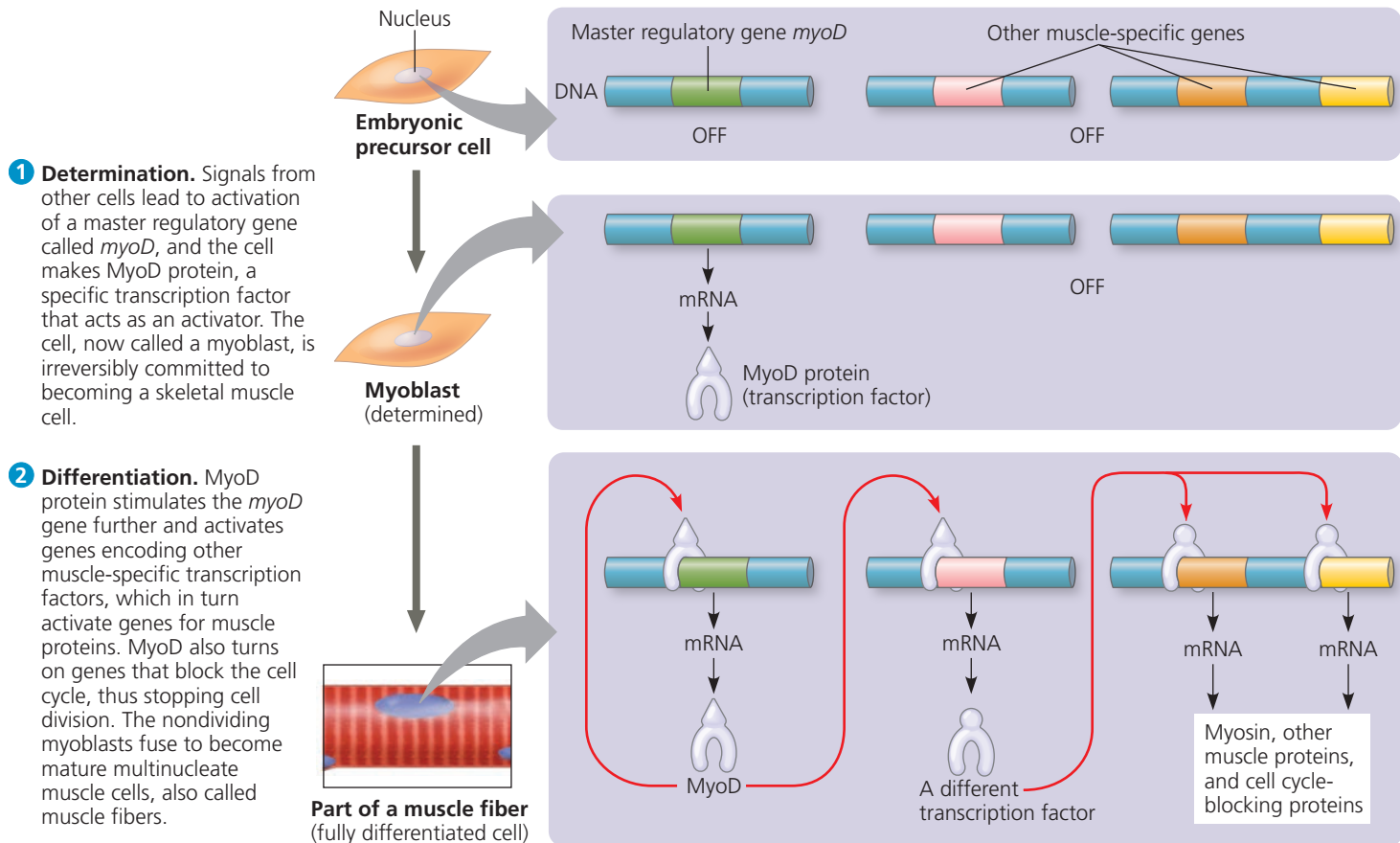
Muscle cells develop from embryonic precursor cells that have the potential to develop into a number of cell types, including cartilage cells and fat cells, but particular conditions commit them to becoming muscle cells. Although the committed cells appear unchanged under the microscope, determination has occurred, and they are now *myoblasts*. Eventually, myoblasts start to churn out large amounts of muscle-specific proteins and fuse to form mature, elongated, multinucleate skeletal muscle cells.

Researchers have worked out what happens at the molecular level during muscle cell determination. To do so, they grew embryonic precursor cells in culture and analyzed them using molecular techniques you will learn about in Concepts 19.1 and 19.2. In a series of experiments, they isolated different genes, caused each to be expressed in a separate embryonic precursor cell, and then looked for differentiation into myoblasts and muscle cells. In this way, they identified several so-called “master regulatory genes” whose protein products commit the cells to becoming skeletal muscle. Thus, in the case of muscle cells, the molecular basis of determination is the expression of one or more of these master regulatory genes.

To understand more about how determination occurs in muscle cell differentiation, let's focus on the master regulatory gene called *myoD*. The *myoD* gene deserves its designation as a master regulatory gene. Researchers have shown that the MyoD protein it encodes is capable of changing some kinds of fully differentiated nonmuscle cells, such as fat cells and liver cells, into muscle cells. Why doesn't MyoD work on *all* kinds of cells? One likely explanation is that activation of muscle-specific genes is not solely dependent on MyoD but requires a particular *combination* of regulatory proteins, some of which are lacking in cells that do not respond to MyoD. The determination and differentiation of other kinds of tissues may play out in a similar fashion. A growing body of experimental evidence supports the idea that master regulatory proteins like MyoD might actually function by opening the chromatin in particular regions. This allows access to transcription machinery for activation of the next set of cell-type-specific genes.

What is the molecular basis for muscle cell differentiation? The MyoD protein is a transcription factor (see Figure 18.9) that binds to specific control elements in the enhancers of various target genes and stimulates their expression (Figure 18.18). Some target genes for MyoD encode still other muscle-specific transcription factors. MyoD also stimulates expression of the

▼ **Figure 18.18 Determination and differentiation of muscle cells.** Skeletal muscle cells arise from embryonic cells as a result of changes in gene expression. (In this depiction, the process of gene activation is greatly simplified.)



WHAT IF? ► What would happen if a mutation in the *myoD* gene resulted in the production of an altered MyoD protein that could not activate the *myoD* gene?

myoD gene itself, an example of positive feedback that perpetuates MyoD's effect in maintaining the cell's differentiated state. Presumably, all the genes activated by MyoD have enhancer control elements recognized by MyoD and are thus coordinately controlled. Finally, the secondary transcription factors activate the genes for proteins such as myosin and actin that confer the unique properties of skeletal muscle cells.

We have now seen how different programs of gene expression that are activated in the fertilized egg can result in differentiated cells and tissues. But for the tissues to function effectively in the organism as a whole, the organism's *body plan*—its overall three-dimensional arrangement—must be established and superimposed on the differentiation process. Next we'll investigate the molecular basis for the establishment of the body plan, using the well-studied fruit fly *Drosophila melanogaster* as an example.

Pattern Formation: Setting Up the Body Plan

Cytoplasmic determinants and inductive signals both contribute to the development of a spatial organization in

which the tissues and organs of an organism are all in their characteristic places. This process is referred to as **pattern formation**.

Just as the locations of the front, back, and sides of a new building are determined before construction begins, pattern formation in animals begins in the early embryo, when the major axes of an animal are established. In a bilaterally symmetrical animal, the relative positions of head and tail, right and left sides, and back and front—the three major body axes—are set up before the organs appear. The molecular cues that control pattern formation, collectively called **positional information**, are provided by cytoplasmic determinants and inductive signals (see Figure 18.17). These cues tell a cell its location relative to the body axes and to neighboring cells and determine how the cell and its descendants will respond to future molecular signals.

During the first half of the 20th century, classical embryologists made detailed anatomical observations of embryonic development in a number of species and performed experiments in which they manipulated embryonic tissues. Although this research laid the groundwork for understanding the mechanisms of development, it did not reveal the

specific molecules that guide development or determine how patterns are established.

In the 1940s, scientists began using the genetic approach—the study of mutants—to investigate *Drosophila* development. That approach has had spectacular success. These studies have established that genes control development and have led to an understanding of the key roles that specific molecules play in defining position and directing differentiation. By combining anatomical, genetic, and biochemical approaches to the study of *Drosophila* development, researchers have discovered developmental principles common to many other species, including humans.

The Life Cycle of *Drosophila*

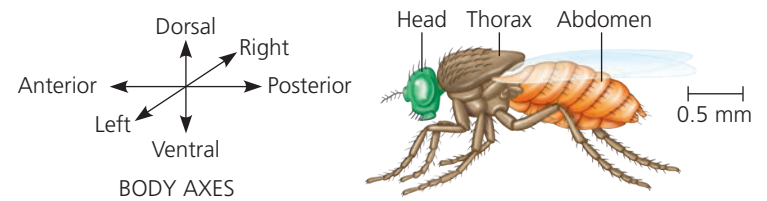
Fruit flies and other arthropods have a modular construction, an ordered series of segments. These segments make up the body's three major parts: the head, the thorax (the mid-body, from which the wings and legs extend), and the abdomen (**Figure 18.19a**). Like other bilaterally symmetrical animals, *Drosophila* has an anterior-posterior (head-to-tail) axis, a dorsal-ventral (back-to-belly) axis, and a right-left axis. In *Drosophila*, cytoplasmic determinants that are localized in the unfertilized egg provide positional information for the placement of anterior-posterior and dorsal-ventral axes even before fertilization. We'll focus here on the molecules involved in establishing the anterior-posterior axis.

The *Drosophila* egg develops in one of the female's ovaries, adjacent to ovarian cells called nurse cells and surrounded by so-called follicle cells (**Figure 18.19b**, top). These support cells supply the egg with nutrients, mRNAs, and other substances needed for development and make the egg shell. After fertilization and laying of the egg, embryonic development results in the formation of a segmented larva, which goes through three larval stages. Then, in a process much like that by which a caterpillar becomes a butterfly, the fly larva forms a pupa in which it metamorphoses into the adult fly pictured in **Figure 18.19a**.

Genetic Analysis of Early Development: Scientific Inquiry

Edward B. Lewis was a visionary American biologist who, in the 1940s, first showed the value of the genetic approach to studying embryonic development in *Drosophila*. Lewis studied bizarre mutant flies with developmental defects that led to extra wings or legs in the wrong place (**Figure 18.20**). He located the mutations on the fly's genetic map, thus connecting the developmental abnormalities to specific genes. This research supplied the first concrete evidence that genes somehow direct the developmental processes studied by embryologists. The genes Lewis discovered, called **homeotic genes**, are regulatory genes that control pattern formation in the late embryo, larva, and adult.

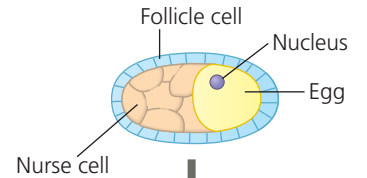
▼ **Figure 18.19** Key events in the *Drosophila* life cycle.



(a) Adult. The adult fly is segmented, and multiple segments make up each of the three main body parts—head, thorax, and abdomen. The body axes are shown by arrows.

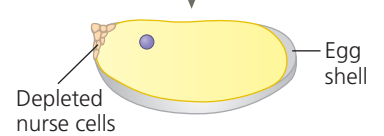
1 Developing egg

within one ovarian follicle (among many in an ovary). The egg (yellow) is surrounded by support cells (follicle cells).



2 Mature, unfertilized egg.

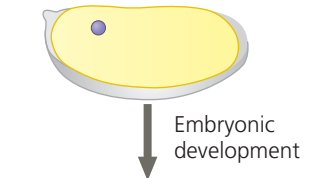
The developing egg enlarges as nutrients and mRNAs are supplied to it by other support cells (nurse cells), which shrink. Eventually, the mature egg fills the egg shell that is secreted by the follicle cells.



Fertilization
Laying of egg

3 Fertilized egg.

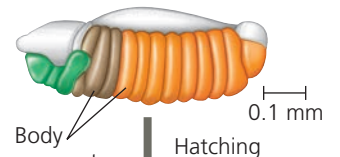
The egg is fertilized within the mother and then laid.



Embryonic
development

4 Segmented embryo.

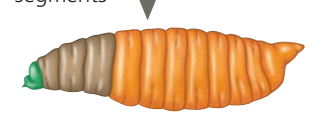
The egg develops into a segmented embryo.



Hatching

5 Larva.

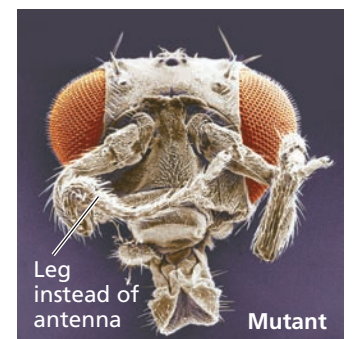
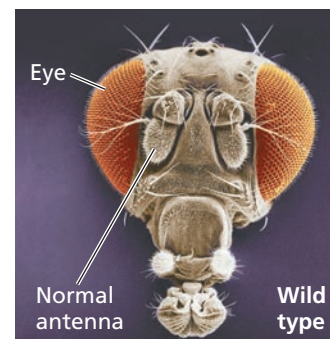
The embryo develops into a larva, which has three stages. The third stage forms a pupa (not shown), within which the larva metamorphoses into the adult shown in (a).



(b) Development from egg to larva.

▼ **Figure 18.20** Abnormal pattern formation in *Drosophila*.

Mutations in homeotic genes cause abnormal placement of structures in an animal, such as the legs extending from the mutant fly's head in place of antenna. (Colorized SEMs)



by embryologists a century ago, in which gradients of substances called **morphogens** establish an embryo's axes and other features of its form.

DNA technology and other modern biochemical methods enabled the researchers to test whether the *bicoid* product, a protein called Bicoid, is in fact a morphogen that determines the anterior end of the fly. The first question they asked was whether the mRNA and protein products of this gene are located in the egg in a position consistent with the hypothesis. They found that *bicoid* mRNA is highly concentrated at the extreme anterior end of the mature egg (**Figure 18.22**). After the egg is fertilized, the mRNA is translated into protein. The Bicoid protein then diffuses from the anterior end toward the posterior, resulting in a gradient of protein within the early embryo, with the highest concentration at the anterior end. These results are consistent with the hypothesis that Bicoid protein specifies the fly's anterior end. To test the hypothesis more specifically, scientists injected pure *bicoid* mRNA into various regions of early embryos. The protein that resulted from its translation caused anterior structures to form at the injection sites.

The *bicoid* research was groundbreaking for several reasons. First, it led to the identification of a specific protein required for some of the earliest steps in pattern formation. It thus helped us understand how different regions of the egg can give rise to cells that go down different developmental pathways. Second, it increased our understanding of the mother's critical role in the initial phases of embryonic development. Finally, the principle that a gradient of morphogens can determine polarity and position has proved to be a key developmental concept for a number of species, just as early embryologists had hypothesized.

Maternal mRNAs are crucial during development of many species. In *Drosophila*, gradients of specific proteins encoded by maternal mRNAs not only determine the posterior and anterior ends but also establish the dorsal-ventral axis. As the fly embryo grows, it reaches a point when the embryonic program of gene expression takes over, and the maternal mRNAs must be destroyed. (This process involves miRNAs in *Drosophila* and other species.) Later, positional information encoded by the embryo's genes, operating on an ever finer scale, establishes a specific number of correctly oriented segments and triggers the formation of each segment's characteristic structures. When the genes operating in this final step are abnormal, the pattern of the adult is abnormal, as you saw in Figure 18.20.

MB Interview with Nancy Hopkins: Studying the genetic basis of development in zebrafish

Evolutionary Developmental Biology ("Evo-Devo")

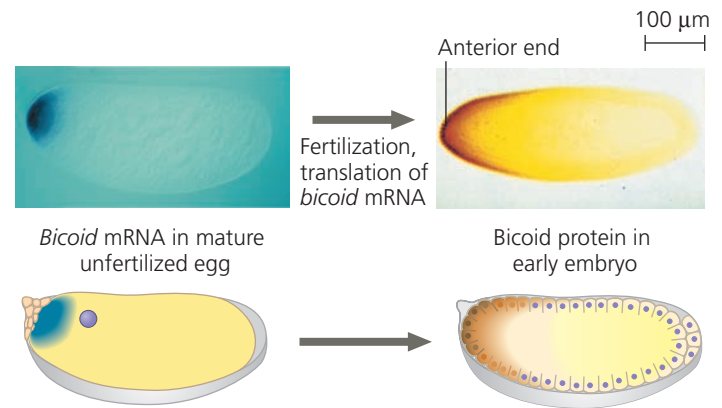
EVOLUTION The fly with legs emerging from its head in Figure 18.20 is the result of a single mutation in one gene, a homeotic gene. The gene does not encode any antenna

▼ Figure 18.22

Inquiry Could Bicoid be a morphogen that determines the anterior end of a fruit fly?

Experiment Using a genetic approach to study *Drosophila* development, Christiane Nüsslein-Volhard and colleagues at two research institutions in Germany analyzed expression of the *bicoid* gene. The researchers hypothesized that *bicoid* normally codes for a morphogen that specifies the head (anterior) end of the embryo. To begin to test this hypothesis, they used molecular techniques to determine whether the mRNA and protein encoded by this gene were found in the anterior end of the fertilized egg and early embryo of wild-type flies.

Results *Bicoid* mRNA (dark blue in the light micrographs and drawings) was confined to the anterior end of the unfertilized egg. Later in development, Bicoid protein (dark orange) was seen to be concentrated in cells at the anterior end of the embryo.



Conclusion The location of *bicoid* mRNA and the diffuse gradient of Bicoid protein seen later are consistent with the hypothesis that Bicoid protein is a morphogen specifying formation of head-specific structures.

Further Reading C. Nüsslein-Volhard et al., Determination of anteroposterior polarity in *Drosophila*, *Science* 238:1675–1681 (1987); W. Driever and C. Nüsslein-Volhard, A gradient of Bicoid protein in *Drosophila* embryos, *Cell* 54:83–93 (1988); T. Berleth et al., The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo, *EMBO Journal* 7:1749–1756 (1988).

WHAT IF? > The researchers needed further evidence, so they injected *bicoid* mRNA into the anterior end of an egg from a female with a mutation disabling the *bicoid* gene. Given that the hypothesis was supported, what must their results have been?

MB Animation: Role of *bicoid* Gene in *Drosophila* Development

protein, however. Instead, it encodes a transcription factor that regulates other genes, and its malfunction leads to misplaced structures, such as legs instead of antennae. The observation that a change in gene regulation during development could lead to such a fantastic change in body form prompted some scientists to consider whether these types of mutations could contribute to evolution by generating novel body shapes. Ultimately, this line of inquiry gave rise to the field of evolutionary developmental biology, so-called “evo-devo,” which will be further discussed in Concept 20.6.

In this section, we have seen how a carefully orchestrated program of sequential gene regulation controls the

transformation of a fertilized egg into a multicellular organism. The program is carefully balanced between turning on the genes for differentiation in the right place and turning off other genes. Even when an organism is fully developed, gene expression is regulated in a similarly fine-tuned manner. In the final section of the chapter, we'll consider how fine this tuning is by looking at how specific changes in expression of just a few genes can lead to the development of cancer.

CONCEPT CHECK 18.4

1. **MAKE CONNECTIONS** > As you learned in Chapter 12, mitosis gives rise to two daughter cells that are genetically identical to the parent cell. Yet you, the product of many mitotic divisions, are not composed of identical, zygote-like cells. Why?
2. **MAKE CONNECTIONS** > Explain how the signaling molecules released by an embryonic cell can induce changes in a neighboring cell without entering the cell. (See Figures 9.15 and 9.16.)
3. How do fruit fly maternal effect genes determine the polarity of the egg and the embryo?
4. **WHAT IF?** > In Figure 18.17b, the lower cell is synthesizing signaling molecules, whereas the upper cell is expressing receptors for these molecules. In terms of gene regulation and cytoplasmic determinants, explain how these cells came to synthesize different molecules.

For suggested answers, see Appendix A.

CONCEPT 18.5

Cancer results from genetic changes that affect cell cycle control

In Concept 12.3, we considered cancer as a type of disease in which cells escape from the control mechanisms that normally limit their growth. Now that we have discussed the molecular basis of gene expression and its regulation, we are ready to look

at cancer more closely. The gene regulation systems that go wrong during cancer turn out to be the very same systems that play important roles in embryonic development, the immune response, and many other biological processes. Thus, research into the molecular basis of cancer has both benefited from and informed many other fields of biology.

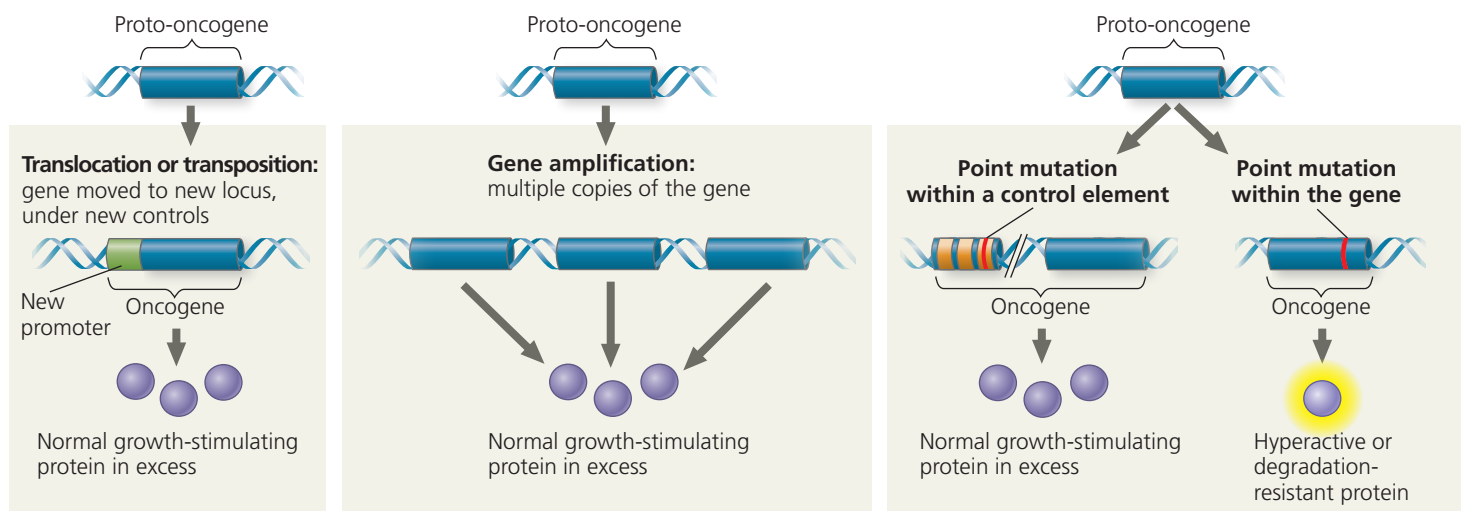
Types of Genes Associated with Cancer

The genes that normally regulate cell growth and division during the cell cycle include genes for growth factors, their receptors, and the intracellular molecules of signaling pathways. (To review cell signaling, see Concept 9.2; for regulation of the cell cycle, see Concept 12.3.) Mutations that alter any of these genes in somatic cells can lead to cancer. The agent of such change can be random spontaneous mutation. However, it is also likely that many cancer-causing mutations result from environmental influences, such as chemical carcinogens, X-rays and other high-energy radiation, and some viruses.

Cancer research led to the discovery of cancer-causing genes called **oncogenes** (from the Greek *onco*, tumor) in certain types of viruses. Subsequently, close counterparts of viral oncogenes were found in the genomes of humans and other animals. The normal versions of the cellular genes, called **proto-oncogenes**, code for proteins that stimulate normal cell growth and division.

How might a proto-oncogene—a gene that has an essential function in normal cells—become an oncogene, a cancer-causing gene? In general, an oncogene arises from a genetic change that leads to an increase either in the amount of the proto-oncogene's protein product or in the intrinsic activity of each protein molecule. The genetic changes that convert proto-oncogenes to oncogenes fall into three main categories: movement of DNA within the genome, amplification of a proto-oncogene, and point mutations in a control element or in the proto-oncogene itself (**Figure 18.23**).

▼ **Figure 18.23** Genetic changes that can turn proto-oncogenes into oncogenes.



Cancer cells are frequently found to contain chromosomes that have broken and rejoined incorrectly, translocating fragments from one chromosome to another (see Figure 15.14). Having learned how gene expression is regulated, you can now see the possible consequences of such translocations. If a translocated proto-oncogene ends up near an especially active promoter (or other control element), its transcription may increase, making it an oncogene. The second main type of genetic change, amplification, increases the number of copies of the proto-oncogene in the cell through repeated gene duplication (discussed in Concept 20.5). The third possibility is a point mutation either in the promoter or an enhancer that controls a proto-oncogene, causing an increase in its expression, or in the coding sequence of the proto-oncogene, changing the gene's product to a protein that is more active or more resistant to degradation than the normal protein. These mechanisms can lead to abnormal stimulation of the cell cycle and put the cell on the path to becoming a cancer cell.

In addition to genes whose products normally promote cell division, cells contain genes whose normal products *inhibit* cell division. Such genes are called **tumor-suppressor genes** since the proteins they encode help prevent uncontrolled cell growth. Any mutation that decreases the normal activity of a tumor-suppressor protein may contribute to the onset of cancer, in effect stimulating growth through the absence of suppression.

The protein products of tumor-suppressor genes have various functions. Some repair damaged DNA, a function that

prevents the cell from accumulating cancer-causing mutations. Other tumor-suppressor proteins control the adhesion of cells to each other or to the extracellular matrix; proper cell anchorage is crucial in normal tissues—and is often absent in cancers. Still other tumor-suppressor proteins are components of cell-signaling pathways that inhibit the cell cycle.

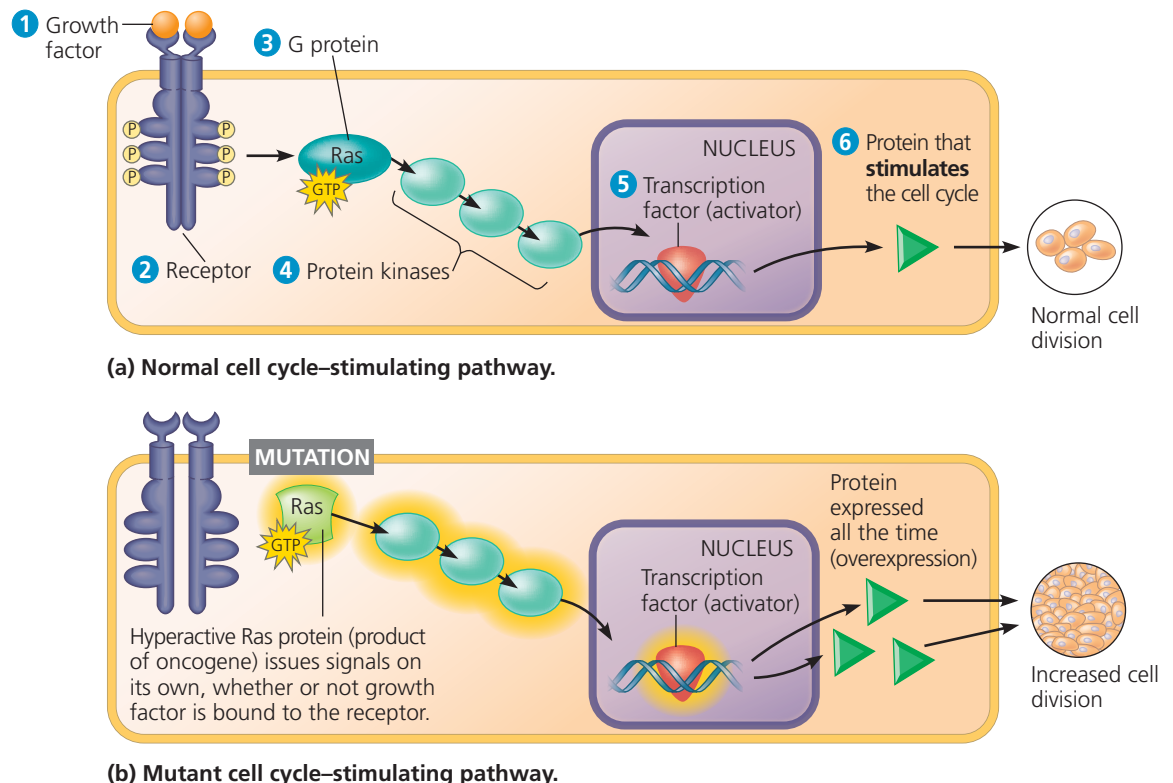
Interference with Normal Cell-Signaling Pathways

The proteins encoded by many proto-oncogenes and tumor-suppressor genes are components of cell-signaling pathways. Let's take a closer look at how such proteins function in normal cells and what goes wrong with their function in cancer cells. We will focus on the products of two key genes, the *ras* proto-oncogene and the *p53* tumor-suppressor gene. Mutations in *ras* occur in about 30% of human cancers, and mutations in *p53* in more than 50%.

The Ras protein, encoded by the ***ras* gene** (named for *rat sarcoma*, a connective tissue cancer), is a G protein that relays a signal from a growth factor receptor on the plasma membrane to a cascade of protein kinases (see Figures 9.8 and 9.10). The cellular response at the end of the pathway is the synthesis of a protein that stimulates the cell cycle (**Figure 18.24a**). Normally, such a pathway will not operate unless triggered by the appropriate growth factor. But certain mutations in the *ras* gene can lead to production of a hyperactive Ras protein that triggers the kinase cascade even in

► Figure 18.24 Normal and mutant cell cycle-stimulating pathway.

(a) The normal pathway is triggered by 1 a growth factor that binds to 2 its receptor in the plasma membrane. The signal is relayed to 3 a G protein called Ras. Like all G proteins, Ras is active when GTP is bound to it. Ras passes the signal to 4 a series of protein kinases. The last kinase activates 5 a transcription factor (activator) that turns on one or more genes for 6 a protein that stimulates the cell cycle. (b) If a mutation makes Ras or any other pathway component abnormally active, excessive cell division and cancer may result.



the absence of growth factor, resulting in increased cell division (**Figure 18.24b**). In fact, hyperactive versions or excess amounts of any of the pathway's components can have the same outcome: excessive cell division.

Figure 18.25a shows a pathway in which an intracellular signal leads to the synthesis of a protein that suppresses the cell cycle. In this case, the signal is damage to the cell's DNA, perhaps as the result of exposure to ultraviolet light. Operation of this signaling pathway blocks the cell cycle until the damage has been repaired. Otherwise, the damage might contribute to tumor formation by causing mutations or chromosomal abnormalities. Thus, the genes for the components of the pathway act as tumor-suppressor genes. The **p53 gene**, named for the 53,000-dalton molecular weight of its protein product, is a tumor-suppressor gene. The protein it encodes is a specific transcription factor that promotes the synthesis of cell cycle-inhibiting proteins. That is why a mutation that knocks out the p53 gene, like a mutation that leads to a hyperactive Ras protein, can lead to excessive cell growth and cancer (**Figure 18.25b**).

The p53 gene has been called the “guardian angel of the genome.” Once the gene is activated—for example, by DNA damage—the p53 protein functions as an activator for several other genes. Often it activates a gene called p21, whose product halts the cell cycle by binding to cyclin-dependent kinases, allowing time for the cell to repair the DNA. Researchers recently showed that p53 also activates

expression of a group of miRNAs, which in turn inhibit the cell cycle. In addition, the p53 protein can turn on genes directly involved in DNA repair. Finally, when DNA damage is irreparable, p53 activates “suicide” genes, whose protein products bring about programmed cell death (apoptosis; see Figure 9.20). Thus, p53 acts in several ways to prevent a cell from passing on mutations due to DNA damage. If mutations do accumulate and the cell survives through many divisions—as is more likely if the p53 tumor-suppressor gene is defective or missing—cancer may ensue. The many functions of p53 suggest a complex picture of regulation in normal cells, one that we do not yet fully understand.

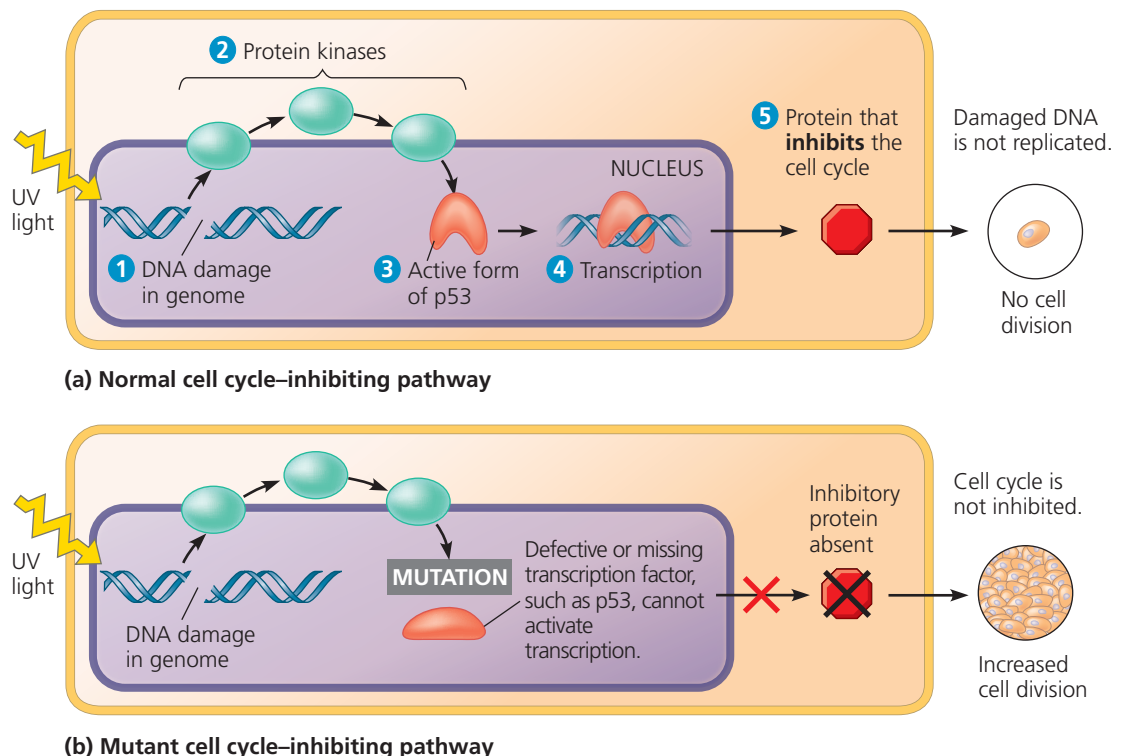
A recent study may underscore the protective role of p53 while illuminating a long-standing research question: Why is cancer so rare among elephants? The incidence of cancer among elephants in zoo-based studies has been estimated at about 3%, compared to closer to 30% for humans. Genome sequencing revealed that elephants have 20 copies of the p53 gene, compared to one copy in humans, other mammals, and even manatees, elephants' closest living relatives. There are undoubtedly other underlying reasons, but the correlation between low cancer rate and extra copies of the p53 gene bears further investigation.

For the present, the diagrams in Figure 18.24 and Figure 18.25 are an accurate view of how mutations can contribute to cancer, but we still don't know exactly how a particular cell becomes a cancer cell. As we discover previously unknown

► **Figure 18.25 Normal and mutant cell cycle-inhibiting pathway.**

(a) In the normal pathway, **1** DNA damage is an intracellular signal that is passed via **2** protein kinases, leading to activation of **3** p53. Activated p53 promotes **4** transcription of the gene for **5** a protein that inhibits the cell cycle. The resulting suppression of cell division ensures that the damaged DNA is not replicated. If the DNA damage is irreparable, then the p53 signal leads to programmed cell death (apoptosis). **(b)** Mutations causing deficiencies in any pathway component can contribute to the development of cancer.

? Explain whether a cancer-causing mutation in a tumor-suppressor gene, such as p53, is more likely to be a recessive or a dominant mutation.



aspects of gene regulation, it is informative to study their role in the onset of cancer. Such studies have shown, for instance, that DNA methylation and histone modification patterns differ in normal and cancer cells and that miRNAs probably participate in cancer development. While we've learned a lot about cancer by studying cell-signaling pathways, there is still much more to learn.

The Multistep Model of Cancer Development

More than one somatic mutation is generally needed to produce all the changes characteristic of a full-fledged cancer cell. This may help explain why the incidence of cancer increases greatly with age. If cancer results from an accumulation of mutations and if mutations occur throughout life, then the longer we live, the more likely we are to develop cancer.

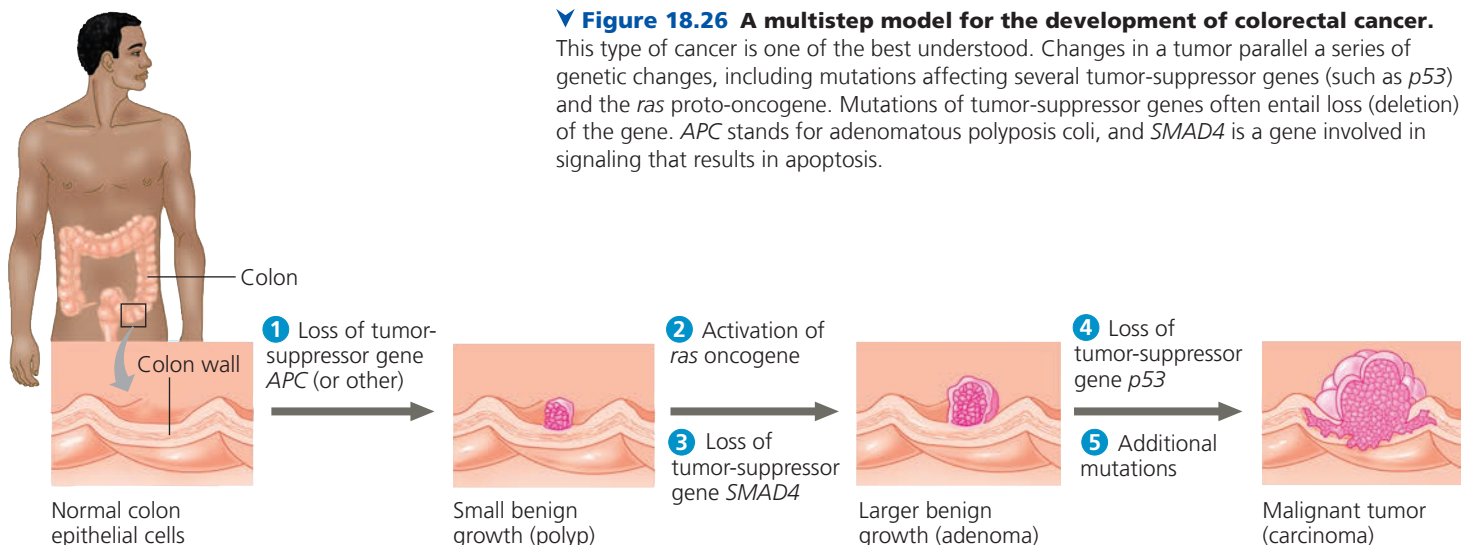
The model of a multistep path to cancer is well supported by studies of one of the best-understood types of human cancer: colorectal cancer, which affects the colon and/or rectum. About 140,000 new cases of colorectal cancer are diagnosed each year in the United States, and the disease causes 50,000 deaths per year. Like most cancers, colorectal cancer develops gradually (**Figure 18.26**). The first sign is often a polyp, a small, benign growth in the colon lining. The cells of the polyp look normal, although they divide unusually frequently. The tumor grows and may eventually become malignant, invading other tissues. The development of a malignant tumor is paralleled by a gradual accumulation of mutations that convert proto-oncogenes to oncogenes and knock out tumor-suppressor genes. A *ras* oncogene and a mutated *p53* tumor-suppressor gene are often involved.

About half a dozen changes must occur at the DNA level for a cell to become fully cancerous. These changes usually

include the appearance of at least one active oncogene and the mutation or loss of several tumor-suppressor genes. Furthermore, since mutant tumor-suppressor alleles are usually recessive, in most cases mutations must knock out *both* alleles in a cell's genome to block tumor suppression. (Most oncogenes, on the other hand, behave as dominant alleles.)

Since we understand the progression of this type of cancer, routine screenings (colonoscopies, for example) are recommended to identify and remove any suspicious polyps. The colorectal cancer mortality rate has been declining for the past 20 years due to increased screening and improved treatments. Treatments for other cancers have improved as well. Advances in the sequencing of DNA and mRNA allow medical researchers to compare the genes expressed by different types of tumors and by the same type in different people. These comparisons have led to personalized treatments based on the molecular characteristics of a person's tumor.

Breast cancer is the second most common form of cancer in the United States, and the first among women. Each year, this cancer strikes over 230,000 women (and some men) in the United States and kills 40,000 (450,000 worldwide). A major problem with understanding breast cancer is its heterogeneity: Tumors differ in significant ways. Identifying differences between types of breast cancer is expected to improve treatment and decrease the mortality rate. In 2012, The Cancer Genome Atlas Network, sponsored by the National Institutes of Health, published the results of a multi-team effort that used a genomics approach to profile subtypes of breast cancer based on their molecular signatures. Four major types of breast cancer were identified (**Figure 18.27**). It is now routine to screen for the presence of particular signaling receptors in any breast cancer tumors, and individuals with breast cancer, along with their physicians, can now make more informed decisions about their treatments.



Genomics, Cell Signaling, and Cancer

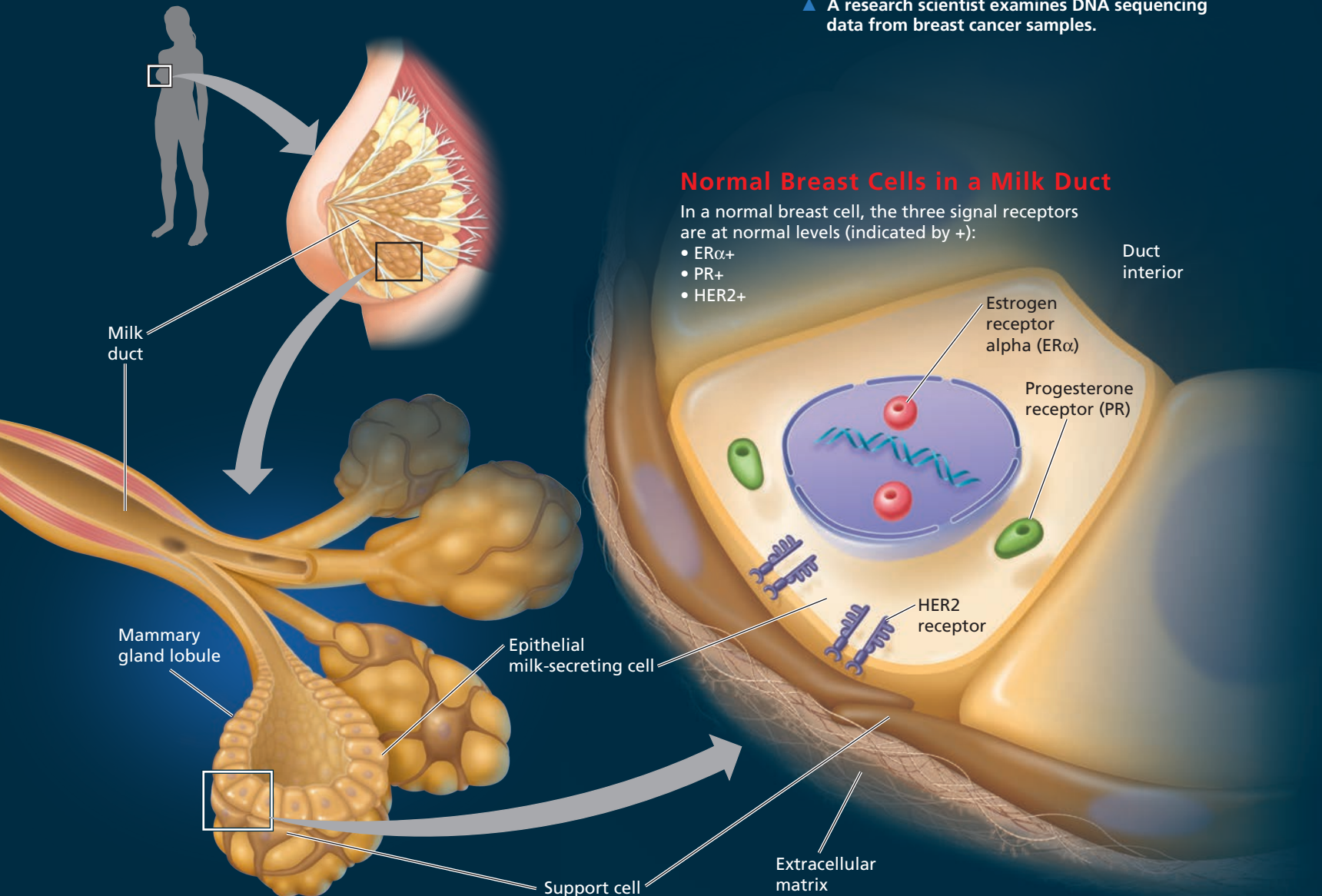
Modern medicine that melds genome-wide molecular studies with cell-signaling research is transforming the treatment of many diseases, such as breast cancer. Using microarray analysis (see Figure 19.13) and other techniques, researchers measured the relative levels of mRNA transcripts for every gene in hundreds of breast cancer tumor samples. They identified four major subtypes of breast cancer that differ in their expression of three signal receptors involved in regulating cell growth and division:

- Estrogen receptor alpha ($ER\alpha$)
- Progesterone receptor (PR)
- HER2, a type of receptor called a receptor tyrosine kinase (see Figure 9.8)

($ER\alpha$ and PR are steroid receptors; see Figure 9.9.) The absence or excess expression of these receptors can cause aberrant cell signaling, leading in some cases to inappropriate cell division, which may contribute to cancer (see Figure 18.24).



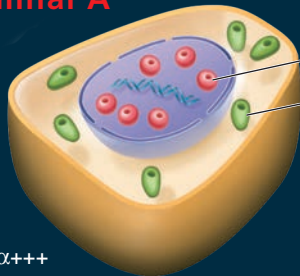
▲ A research scientist examines DNA sequencing data from breast cancer samples.



Breast Cancer Subtypes

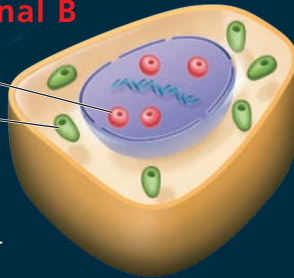
Each breast cancer subtype is characterized by the overexpression (indicated by ++ or +++) or absence (–) of three signal receptors: ER α , PR, and HER2. Breast cancer treatments are becoming more effective because they can be tailored to the specific cancer subtype.

Luminal A



- ER α +++
- PR++
- HER2–
- 40% of breast cancers
- Best prognosis

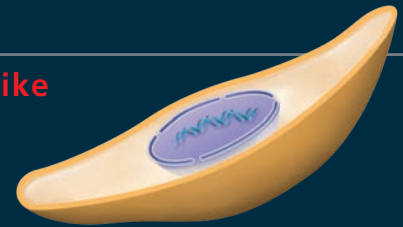
Luminal B



- ER α ++
- PR++
- HER2– (shown here); some HER2++
- 15–20% of breast cancers
- Poorer prognosis than luminal A subtype

Both luminal subtypes overexpress ER α (luminal A more than luminal B) and PR, and usually lack expression of HER2. Both can be treated with drugs that target ER α and inactivate it, the most well-known drug being tamoxifen. These subtypes can also be treated with drugs that inhibit estrogen synthesis.

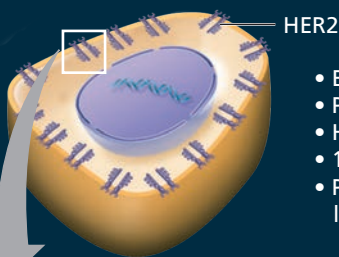
Basal-like



- ER α –
- PR–
- HER2–
- 15–20% of breast cancers
- More aggressive; poorer prognosis than other subtypes

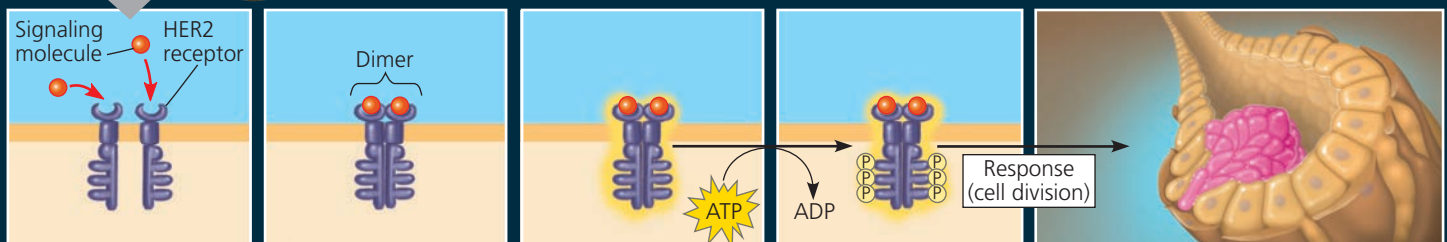
The basal-like subtype is "triple negative"—it does not express ER α , PR, or HER2. It often has a mutation in the tumor-suppressor gene *BRCA1* (see Concept 18.5). Treatments that target ER, PR, or HER2 are not effective, but new treatments are being developed. Currently, patients are treated with cytotoxic chemotherapy, which selectively kills fast-growing cells.

HER2



- ER α –
- PR–
- HER2++
- 10–15% of breast cancers
- Poorer prognosis than luminal A subtype

The HER2 subtype overexpresses HER2. Because it does not express either ER α or PR at normal levels, the cells are unresponsive to therapies that target those two receptors. However, patients with the HER2 subtype can be treated with Herceptin, an antibody protein that inactivates HER2 (see Concept 12.3).



1 Signaling molecules (such as a growth factor) bind to HER2 receptor monomers (single receptor proteins).

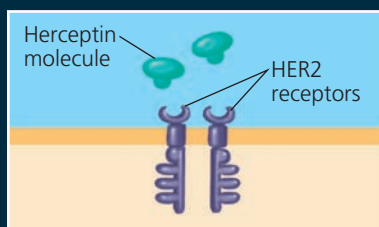
2 Binding of signaling molecules causes two receptor monomers to associate closely with each other, forming a dimer.

3 Formation of a dimer activates each monomer.

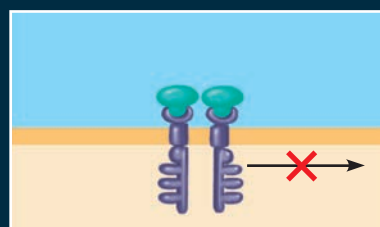
4 Each monomer adds phosphate from ATP to the other monomer, triggering a signal transduction pathway.

5 The signal is transduced through the cell, which leads to a cellular response—in this case, turning on genes that trigger cell division. HER2 cells have up to 100 times as many HER2 receptors as normal cells, so they undergo uncontrolled cell division.

Treatment with Herceptin for the HER2 subtype



1 The drug Herceptin binds to HER2 receptors in place of the usual signaling molecules.



2 In certain patients with the HER2 subtype, signaling is blocked and excessive cell division does not occur.

MAKE CONNECTIONS > When researchers compared gene expression in normal breast cells and cells from breast cancers, they found that the genes showing the most significant differences in expression encoded signal receptors, as shown here. Given what you learned in Chapters 9, 12, and this chapter, explain why this result is not surprising.

Inherited Predisposition and Environmental Factors Contributing to Cancer

The fact that multiple genetic changes are required to produce a cancer cell helps explain the observation that cancers can run in families. An individual inheriting an oncogene or a mutant allele of a tumor-suppressor gene is one step closer to accumulating the necessary mutations for cancer to develop than is an individual without any such mutations.

Geneticists are working to identify inherited cancer alleles so that predisposition to certain cancers can be detected early in life. About 15% of colorectal cancers, for example, involve inherited mutations. Many of these affect the tumor-suppressor gene called *adenomatous polyposis coli*, or *APC* (see Figure 18.26). This gene has multiple functions in the cell, including regulation of cell migration and adhesion. Even in patients with no family history of the disease, the *APC* gene is mutated in 60% of colorectal cancers. In these individuals, new mutations must occur in both *APC* alleles before the gene's function is lost. Since only 15% of colorectal cancers are associated with known inherited mutations, researchers continue to try to identify “markers” that could predict the risk of developing this type of cancer.

Given the prevalence and significance of breast cancer, it is not surprising that it was one of the first cancers for which the role of inheritance was investigated. It turns out that for 5–10% of patients with breast cancer, there is evidence of a strong inherited predisposition. Geneticist Mary-Claire King began working on this problem in the mid-1970s. After 16 years of research, she convincingly demonstrated that mutations in one gene—*BRCA1*—were associated with increased susceptibility to breast cancer, a finding that flew in the face of medical opinion at the time. (*BRCA* stands for breast cancer.) Mutations in that gene or a gene called *BRCA2* are found in at least half of inherited breast cancers, and tests using DNA sequencing can detect these mutations. A woman who inherits one mutant *BRCA1* allele has a 60% probability of developing breast cancer before the age of 50, compared with only a 2% probability for an individual homozygous for the normal allele.

BRCA1 and *BRCA2* are considered tumor-suppressor genes because their wild-type alleles protect against breast cancer and their mutant alleles are recessive. (Note that mutations in *BRCA1* are commonly found in the genomes of cells from basal-like breast cancers; see Figure 18.27.) The *BRCA1* and *BRCA2* proteins both appear to function in the cell's DNA damage repair pathway. More is known about *BRCA2*, which, along with another protein, helps repair breaks that occur in both strands of DNA; this repair function is crucial for maintaining undamaged DNA.



Interview with Mary-Claire King: Discovering breast cancer genes

Because DNA breakage can contribute to cancer, it makes sense that the risk of cancer can be lowered by minimizing exposure to DNA-damaging agents, such as the ultraviolet radiation in sunlight and chemicals found in cigarette smoke. Novel genomics-based analyses of specific cancers, such as the approach described in Figure 18.27, are contributing to both early diagnosis and development of treatments that interfere with expression of key genes in tumors. Ultimately, such approaches are expected to lower the death rate from cancer.

The Role of Viruses in Cancer

The study of genes associated with cancer, inherited or not, increases our basic understanding of how disruption of normal gene regulation can result in this disease. In addition to the mutations and other genetic alterations described in this section, a number of *tumor viruses* can cause cancer in various animals, including humans. In fact, one of the earliest breakthroughs in understanding cancer came in 1911, when Peyton Rous, an American pathologist, discovered a virus that causes cancer in chickens. The Epstein-Barr virus, which causes infectious mononucleosis, has been linked to several types of cancer in humans, notably Burkitt's lymphoma. Papillomaviruses are associated with cancer of the cervix, and a virus called HTLV-1 causes a type of adult leukemia. Viruses play a role in about 15% of the cases of human cancer.

Viruses may at first seem very different from mutations as a cause of cancer. However, we now know that viruses can interfere with gene regulation in several ways if they integrate their genetic material into the DNA of a cell. Viral integration may donate an oncogene to the cell, disrupt a tumor-suppressor gene, or convert a proto-oncogene to an oncogene. Some viruses produce proteins that inactivate p53 and other tumor-suppressor proteins, making the cell more prone to becoming cancerous. Viruses are powerful biological agents; you'll learn more about them in Chapter 26.



Animation: Causes of Cancer

CONCEPT CHECK 18.5

1. Cancer-promoting mutations are likely to have different effects on the activity of proteins encoded by proto-oncogenes than they do on proteins encoded by tumor-suppressor genes. Explain.
2. Under what circumstances is cancer considered to have a hereditary component?
3. **MAKE CONNECTIONS** > The p53 protein can activate genes involved in apoptosis. Review Concept 9.5, and discuss how mutations in genes coding for proteins that function in apoptosis could contribute to cancer.

For suggested answers, see Appendix A.

18 Chapter Review



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SUMMARY OF KEY CONCEPTS

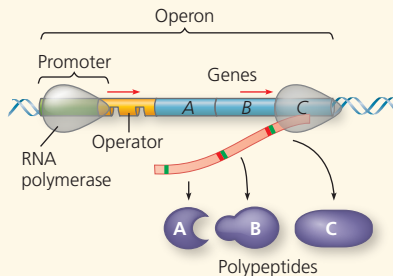
CONCEPT 18.1

Bacteria often respond to environmental change by regulating transcription
(pp. 414–418)



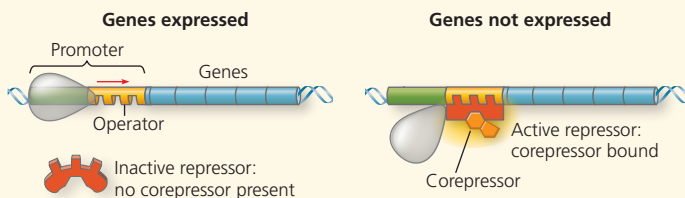
VOCAB
SELF-QUIZ
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- Cells control metabolism by regulating enzyme activity or the expression of genes coding for enzymes. In bacteria, genes are often clustered into **operons**, with one promoter serving several adjacent genes. An **operator** site on the DNA switches the operon on or off, resulting in coordinate regulation of the genes.



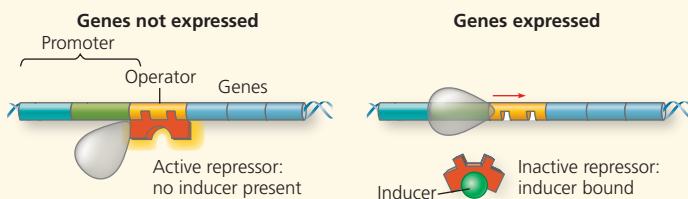
- Both repressible and inducible operons are examples of negative gene regulation. In either type of operon, binding of a specific **repressor** protein to the operator shuts off transcription. (The repressor is encoded by a separate **regulatory gene**.) In a repressible operon, the repressor is active when bound to a **corepressor**, usually the end product of an anabolic pathway.

Repressible operon:



- In an inducible operon, binding of an **inducer** to an innately active repressor inactivates the repressor and turns on transcription. Inducible enzymes usually function in catabolic pathways.

Inducible operon:

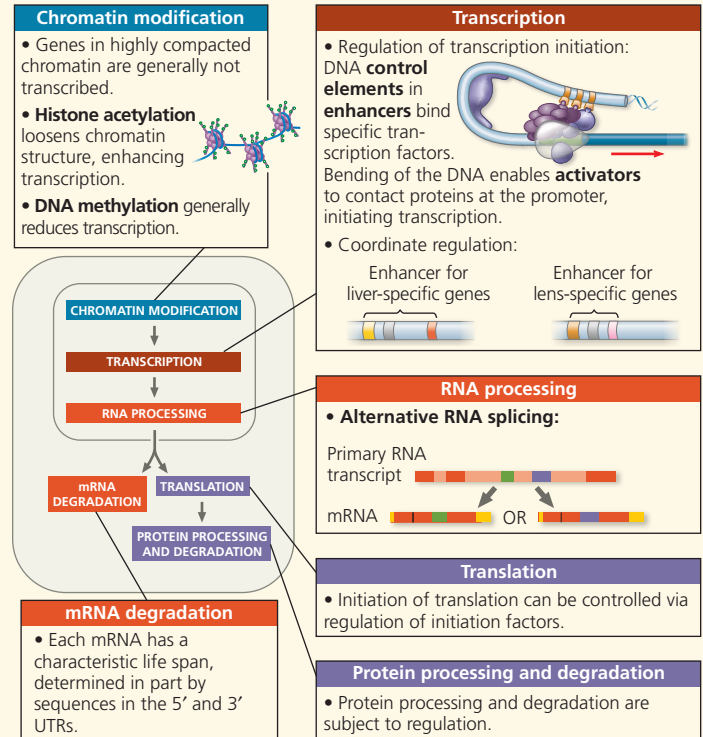


- Some operons are also subject to positive gene regulation via a stimulatory **activator** protein, such as cAMP receptor protein (CRP), which, when activated by **cyclic AMP**, binds to a site within the promoter and stimulates transcription.

? Compare and contrast the roles of a corepressor and an inducer in negative regulation of an operon.

CONCEPT 18.2

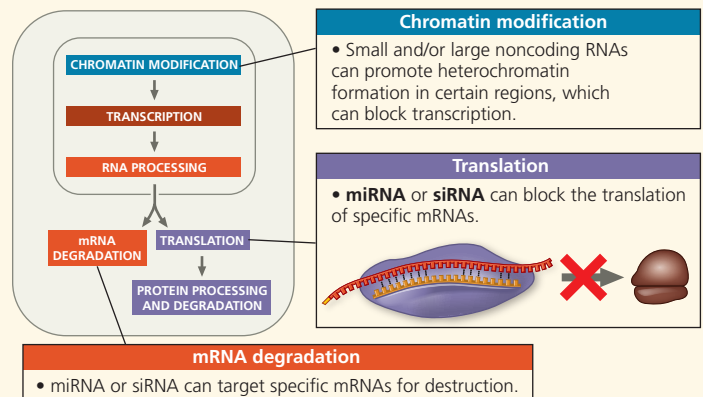
Eukaryotic gene expression is regulated at many stages (pp. 418–426)



? Describe what must happen in a cell for a gene specific to that cell type to be transcribed.

CONCEPT 18.3

Noncoding RNAs play multiple roles in controlling gene expression (pp. 427–429)



? Why are miRNAs called noncoding RNAs? Explain how they participate in gene regulation.

CONCEPT 18.4

A program of differential gene expression leads to the different cell types in a multicellular organism (pp. 429–436)

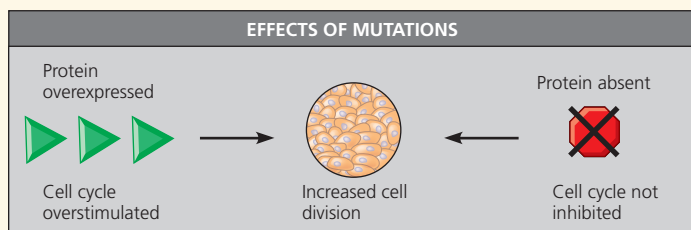
- Embryonic cells become committed to a certain fate (**determination**), and undergo **differentiation**, becoming specialized in structure and function for their determined fate. Cells differ in structure and function not because they contain different genomes but because they express different genes. **Morphogenesis** encompasses the processes that give shape to the organism and its various structures.
- Localized **cytoplasmic determinants** in the unfertilized egg are distributed differentially to daughter cells, where they regulate the expression of those cells' developmental fate. In the process called **induction**, signaling molecules from embryonic cells cause transcriptional changes in nearby target cells.
- Differentiation is heralded by the appearance of tissue-specific proteins, which enable differentiated cells to carry out their specialized roles.
- In animals, **pattern formation**, the development of a spatial organization of tissues and organs, begins in the early embryo. **Positional information**, the molecular cues that control pattern formation, tells a cell its location relative to the body's axes and to other cells. In *Drosophila*, gradients of **morphogens** encoded by **maternal effect genes** determine the body axes. For example, the gradient of **Bicoid** protein determines the anterior-posterior axis.

? Describe the two main processes that cause embryonic cells to head down different pathways to their final fates.

CONCEPT 18.5

Cancer results from genetic changes that affect cell cycle control (pp. 436–442)

- The products of **proto-oncogenes** and **tumor-suppressor genes** control cell division. A DNA change that makes a proto-oncogene excessively active converts it to an **oncogene**, which may promote excessive cell division and cancer. A tumor-suppressor gene encodes a protein that inhibits abnormal cell division. A mutation that reduces the activity of its protein product may lead to excessive cell division and cancer.
- Many proto-oncogenes and tumor-suppressor genes encode components of growth-stimulating and growth-inhibiting signaling pathways, respectively, and mutations in these genes can interfere with normal cell-signaling pathways. A hyperactive version of a protein in a stimulatory pathway, such as **Ras** (a G protein), functions as an oncogene protein. A defective version of a protein in an inhibitory pathway, such as **p53** (a transcription activator), fails to function as a tumor suppressor.



- In the multistep model of cancer development, normal cells are converted to cancer cells by the accumulation of mutations affecting proto-oncogenes and tumor-suppressor genes. Technical advances in DNA and mRNA sequencing are enabling cancer treatments that are more individually based.

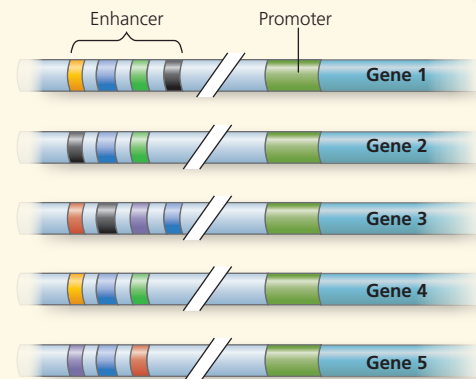
- Genomics-based studies have resulted in researchers proposing four subtypes of breast cancer, based on expression of genes by tumor cells.
- Individuals who inherit a mutant allele of a proto-oncogene or tumor-suppressor gene have a predisposition to develop a particular cancer. Certain viruses promote cancer by integration of viral DNA into a cell's genome.

? Compare the usual functions of proteins encoded by proto-oncogenes with those of proteins encoded by tumor-suppressor genes.

TEST YOUR UNDERSTANDING

Multiple-choice Self-Quiz questions 1–10 can be found in the Study Area in MasteringBiology.

11. **DRAW IT** The diagram below shows five genes, including their enhancers, from the genome of a certain species. Imagine that yellow, blue, green, black, red, and purple activator proteins exist that can bind to the appropriately color-coded control elements in the enhancers of these genes.



- (a) Draw an X above enhancer elements (of all the genes) that would have activators bound in a cell in which only gene 5 is transcribed. Identify which colored activators would be present.
- (b) Draw a dot above all enhancer elements that would have activators bound in a cell in which the green, blue, and yellow activators are present. Identify which gene(s) would be transcribed.
- (c) Imagine that genes 1, 2, and 4 code for nerve-specific proteins, and genes 3 and 5 are skin specific. Identify which activators would have to be present in each cell type to ensure transcription of the appropriate genes.
12. **EVOLUTION CONNECTION** RNAi mechanisms, particularly the expression of miRNAs, are widely found in multicellular eukaryotes, but are not found in several unicellular eukaryotes, such as *Saccharomyces cerevisiae*. On the other hand, the mutation of Dicer, a protein essential for the generation of miRNA, in mice can be lethal for embryos. How can these concepts be reconciled?
13. **SCIENTIFIC INQUIRY** Prostate cells usually require testosterone and other androgens to survive. But some prostate cancer cells thrive despite treatments that eliminate androgens. One hypothesis is that estrogen, often considered a female hormone, may be activating genes normally controlled by an androgen in these cancer cells. Describe one or more experiments to test this hypothesis. (See Figure 9.9 to review the action of these steroid hormones.)

14. SCIENCE, TECHNOLOGY, AND SOCIETY Trace amounts of dioxin were present in Agent Orange, a defoliant sprayed on vegetation during the Vietnam War. Animal tests suggest that dioxin can cause birth defects, cancer, liver and thymus damage, and immune system suppression, sometimes leading to death. But the animal tests are equivocal; a hamster is not affected by a dose that can kill a guinea pig. Dioxin acts like a steroid hormone, entering a cell and binding to a cytoplasmic receptor that then binds the cell's DNA.

- (a) Discuss how this mechanism might help explain the variety of dioxin's effects on different body systems and in different animals.
- (b) Discuss how you might determine whether a type of illness is related to dioxin exposure. Next, discuss how you might determine whether a particular individual became ill as a result of exposure to dioxin. Which would be more difficult to demonstrate? Why?

15. WRITE ABOUT A THEME: INTERACTIONS In a short essay (100–150 words), discuss how the processes shown in Figure 18.2 are examples of feedback mechanisms regulating biological systems in bacterial cells.

16. SYNTHESIZE YOUR KNOWLEDGE



The flashlight fish has an organ under its eye that emits light, which serves to startle predators and attract prey, and allows the fish to communicate with other fish. Some species can rotate the organ inside and then out, so the light appears to flash on and off. The light is actually emitted by bacteria (of the genus *Vibrio*) that live in the organ in a mutualistic relationship with the fish. (The bacteria receive nutrients from the fish.) The bacteria must multiply until they reach a certain density in the organ (a “quorum”; see Concept 9.1), at which point they all begin emitting light at the same time. There is a group of six or so genes, called *lux* genes, whose gene products are necessary for light formation. Given that these bacterial genes are regulated together, propose a hypothesis for how the genes are organized and regulated.

For selected answers, see Appendix A.

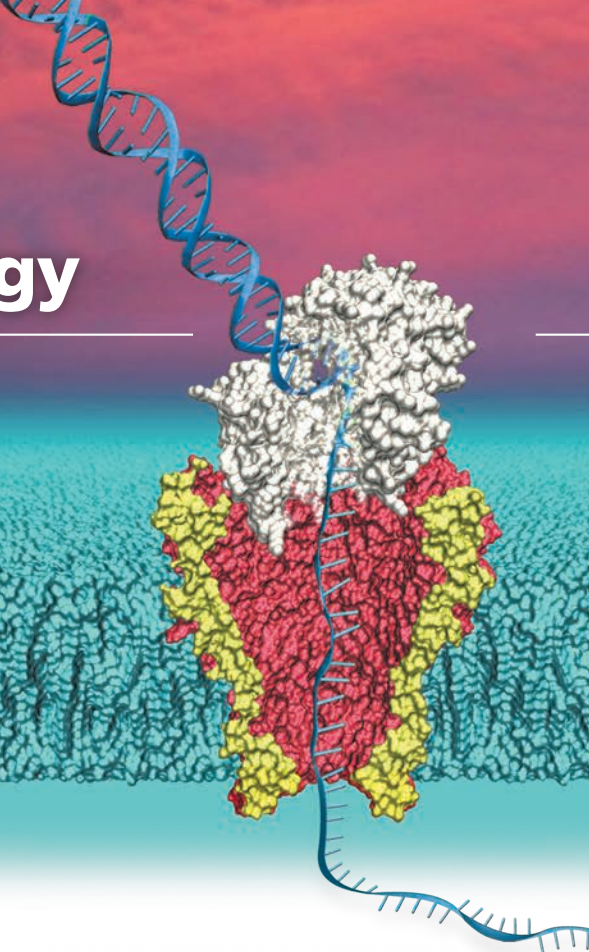


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DNA Technology

19



▲ **Figure 19.1** How can the technique shown in this model advance our sequencing of genomes?

KEY CONCEPTS

- 19.1** DNA sequencing and DNA cloning are valuable tools for genetic engineering and biological inquiry
- 19.2** Biologists use DNA technology to study gene expression and function
- 19.3** Cloned organisms and stem cells are useful for basic research and other applications
- 19.4** The practical applications of DNA-based biotechnology affect our lives in many ways



The DNA Toolbox

The last decade or so has seen some extraordinary feats in biology, among them determination of the complete DNA sequences of several extinct species, including woolly mammoths (see below), Neanderthals, and a 700,000-year-old horse. Pivotal to those discoveries was the sequencing of the human genome, essentially completed in 2003. This endeavor marked a turning point in biology because it sparked remarkable technological advances in DNA sequencing.

The first human genome sequence took several years at a cost of 1 billion dollars; the time and cost of sequencing a genome have been in free fall since then. **Figure 19.1** shows a model of a sequencing technique in which the nucleotides of a single strand of DNA are passed one by one through a very small pore in a membrane, and the resulting tiny changes in an electrical current are used to determine the nucleotide sequence. Developers of this technique, which you will learn more about later in the chapter, claim that ultimately we will be able to sequence a human genome in about 6 hours on a \$900 device the size of a pack of gum.

In this chapter, we'll first describe the main techniques for sequencing and manipulating DNA—**DNA technology**—and for using these DNA tools to analyze gene expression. Next, we'll explore advances in cloning organisms and producing stem cells, techniques that have both expanded our basic understanding of biology

◀ Woolly mammoth, an extinct organism whose genome was sequenced using mummified remains

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Get Ready for This Chapter

and enhanced our ability to apply that understanding to global problems. In the last section, we'll survey the practical applications of DNA-based **biotechnology**, the manipulation of organisms or their components to make useful products. Today, the applications of DNA technology affect everything from agriculture to criminal law to medical research. We will end by considering some of the important social and ethical issues that arise as biotechnology becomes more pervasive in our lives.

CONCEPT 19.1

DNA sequencing and DNA cloning are valuable tools for genetic engineering and biological inquiry

The discovery of the structure of the DNA molecule, and specifically the recognition that its two strands are complementary to each other, opened the door for the development of DNA sequencing and other techniques used in biological research today. Key to these techniques is **nucleic acid hybridization**, the base pairing of one strand of a nucleic acid to a complementary sequence on a strand from a different nucleic acid molecule. In this section, we'll first describe DNA-sequencing techniques. Then we'll explore other important methods used in **genetic engineering**, the direct manipulation of genes for practical purposes.

DNA Sequencing

Researchers can exploit the principle of complementary base pairing to determine the complete nucleotide sequence of a DNA molecule, a process called **DNA sequencing**. The DNA is first cut into fragments, and then each fragment is sequenced. The first automated procedure used a technique called *dideoxyribonucleotide* (or *dideoxy*) *chain termination sequencing*. In this technique, one strand of a DNA fragment is used as a template for synthesis of a nested set of complementary fragments; these are further analyzed to yield the sequence. Biochemist Frederick Sanger received the Nobel Prize in 1980 for developing this method. Dideoxy sequencing is still used for routine small-scale sequencing jobs.



HHMI Video: Sanger Method of DNA Sequencing



In the last 15 years, “next-generation sequencing” techniques have been developed that are much faster (**Figure 19.2**). DNA fragments are amplified (copied) to yield an enormous number of identical fragments (**Figure 19.3**). A specific strand of each fragment is immobilized, and the complementary strand is synthesized, one nucleotide at a time. A chemical technique enables electronic monitors to identify in real time which of the four nucleotides is added; this method is thus called *sequencing by synthesis*. Thousands or hundreds of thousands of fragments,

✓ **Figure 19.2 Next-generation DNA sequencing machines.** These machines use sequencing by synthesis and can sequence 70–90 million nucleotides per hour.



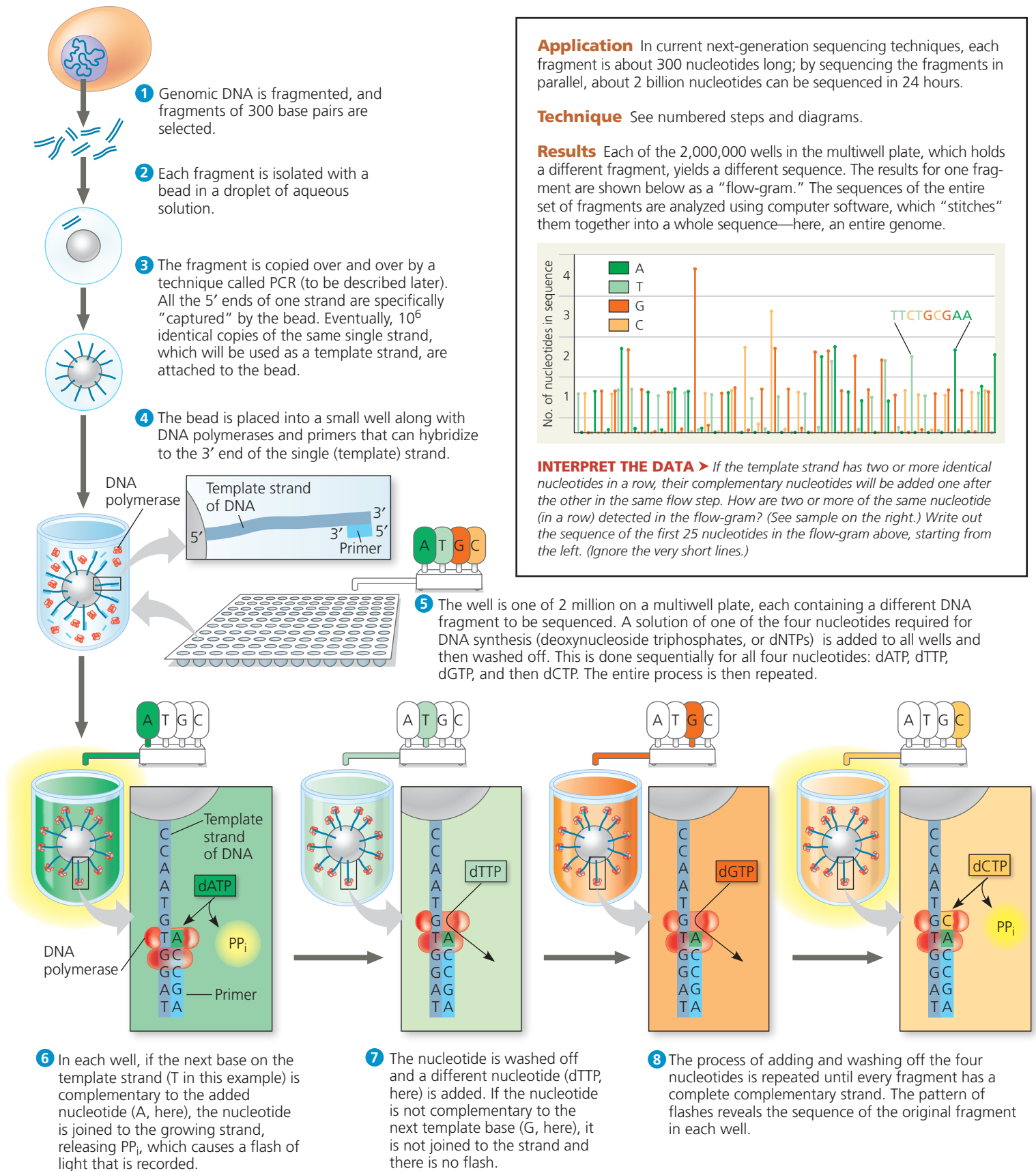
each about 300 nucleotides long, are sequenced in parallel in machines like those shown in Figure 19.2, accounting for the high rate of nucleotides sequenced per hour. This is an example of “high-throughput” DNA technology and is currently the method of choice for studies where massive numbers of DNA samples—even a set of numerous fragments representing an entire genome—are being sequenced.

More and more often, next-generation sequencing is complemented (or in some cases replaced) by “third-generation sequencing,” with each new technique being faster and less expensive than the previous one. In some new methods, the DNA is neither cut into fragments nor amplified. Instead, a single, very long DNA molecule is sequenced on its own. Several groups have developed techniques that move a single strand of a DNA molecule through a very small pore (a *nanopore*) in a membrane, identifying the bases one by one by the distinct way each interrupts an electrical current. One model of this concept is shown in Figure 19.1, in which the pore is a protein channel embedded in a lipid membrane. (Other researchers are using artificial membranes and nanopores.) The idea is that each type of base interrupts the electrical current for a slightly different length of time. In 2015, after a year of use and review by scientists, the first nanopore sequencer went on the market; this device is the size of a small candy bar and connects to a computer via a USB port. Associated software allows immediate identification and analysis of the sequence. This is only one of many approaches to further increase the rate and cut the cost of sequencing, while also allowing the methodology to move out of the laboratory and into the field.

Improved DNA-sequencing techniques have transformed the way in which we can explore fundamental biological questions about evolution and how life works (see Make Connections Figure 5.26). Little more than 15 years after the human genome sequence was announced, researchers had completed the sequencing of thousands of genomes, with tens of thousands in progress. Complete genome sequences have been determined for cells from several cancers, for ancient humans, and for the many bacteria that live in the

▼ **Figure 19.3**

Research Method Sequencing by Synthesis: Next-Generation Sequencing



human intestine. In Chapter 20, you'll learn more about how this rapid acceleration of sequencing technology has revolutionized our study of the evolution of species and the genome itself. Now, let's consider how individual genes are studied.

Making Multiple Copies of a Gene or Other DNA Segment

A molecular biologist studying a particular gene or group of genes faces a challenge. Naturally occurring DNA molecules are very long, and a single molecule usually carries hundreds or even thousands of genes. Moreover, in many eukaryotic genomes, protein-coding genes occupy only a small proportion of the chromosomal DNA, the rest being noncoding nucleotide sequences. A single human gene, for example, might constitute only 1/100,000 of a chromosomal DNA molecule. As a further complication, the distinctions between a gene and the surrounding DNA are subtle, consisting only of differences in nucleotide sequence. To work directly with specific genes, scientists have developed methods for preparing well-defined segments of DNA in multiple identical copies, a process called **DNA cloning**.

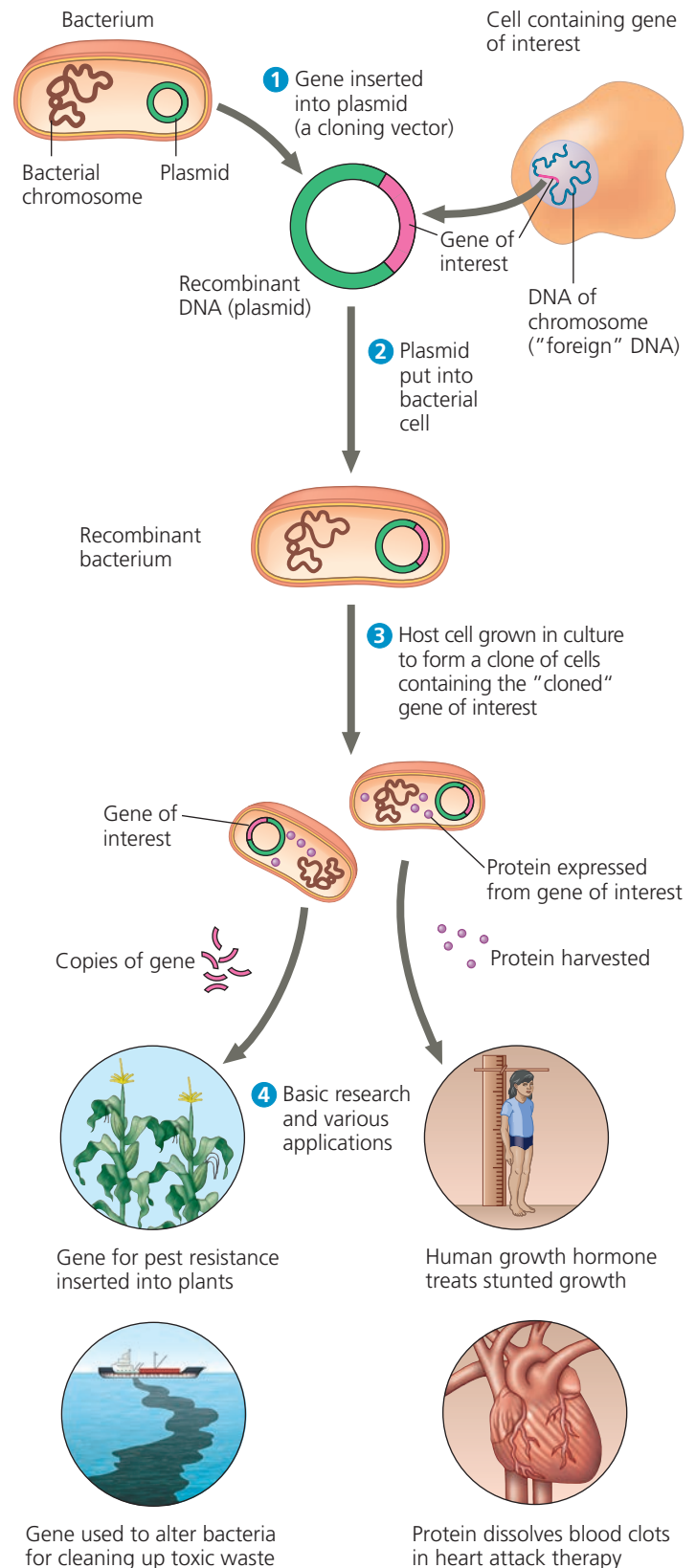
Most methods for cloning pieces of DNA in the laboratory share certain general features. One common approach uses bacteria, most often *Escherichia coli*. Recall from Figure 16.12 that the *E. coli* chromosome is a large, circular molecule of DNA. In addition, *E. coli* and many other bacteria also have **plasmids**, small, circular DNA molecules that are replicated separately. A plasmid has only a small number of genes; these genes may be useful when the bacterium is in a particular environment but may not be required for survival or reproduction under most conditions.

To clone pieces of DNA using bacteria, researchers first obtain a plasmid (originally isolated from a bacterial cell and genetically engineered for efficient cloning) and insert DNA from another source ("foreign" DNA) into it (**Figure 19.4**). The resulting plasmid is now a **recombinant DNA molecule**, a molecule containing DNA from two different sources, very often different species. The plasmid is then returned to a bacterial cell, producing a *recombinant bacterium*. This single cell reproduces through repeated cell divisions to form a clone of cells, a population of genetically identical cells. Because the dividing bacteria replicate the recombinant plasmid and pass it on to their descendants, the foreign DNA and any genes it carries are cloned at the same time. The production of multiple copies of a single gene is a type of DNA cloning called **gene cloning**.

In Figure 19.4, the plasmid acts as a **cloning vector**, a DNA molecule that can carry foreign DNA into a host cell and be replicated there. Bacterial plasmids are widely used as cloning vectors for several reasons: They can be readily obtained from commercial suppliers, manipulated to form recombinant plasmids by insertion of foreign DNA in a test

▼ Figure 19.4 Gene cloning and some uses of cloned genes.

In this simplified diagram of gene cloning, we start with a plasmid (originally isolated from a bacterial cell) and a gene of interest from another organism. Only one plasmid and one copy of the gene of interest are shown at the top of the figure, but the starting materials would include many of each.



tube (referred to as *in vitro*, from the Latin meaning “in glass”), and then easily introduced into bacterial cells. The foreign DNA in Figure 19.4 is a gene from a eukaryotic cell; we will describe in more detail how the foreign DNA segment was obtained later in this section.

Gene cloning is useful for two basic purposes: to make many copies of, or *amplify*, a particular gene and to produce a protein product from it (see Figure 19.4). Researchers can isolate copies of a cloned gene from bacteria for use in basic research or to endow another organism with a new metabolic capability, such as pest resistance. For example, a resistance gene present in one crop species might be cloned and transferred into plants of another species. (Such organisms are called *genetically modified*, or *GM* for short; they will be discussed later in the chapter.) Alternatively, a protein with medical uses, such as human growth hormone, can be harvested in large quantities from cultures of bacteria carrying a cloned gene for the protein. (We’ll describe the techniques for expressing cloned genes later.) Since one gene is only a very small part of the total DNA in a cell, the ability to amplify such rare DNA fragments is crucial for any application involving a single gene.

Using Restriction Enzymes to Make a Recombinant DNA Plasmid

Gene cloning and genetic engineering generally rely on the use of enzymes that cut DNA molecules at a limited number of specific locations. These enzymes, called restriction endonucleases, or **restriction enzymes**, were discovered in the late 1960s by biologists doing basic research on bacteria. Restriction enzymes protect the bacterial cell by cutting up foreign DNA from other organisms or phages (see Concept 26.2).

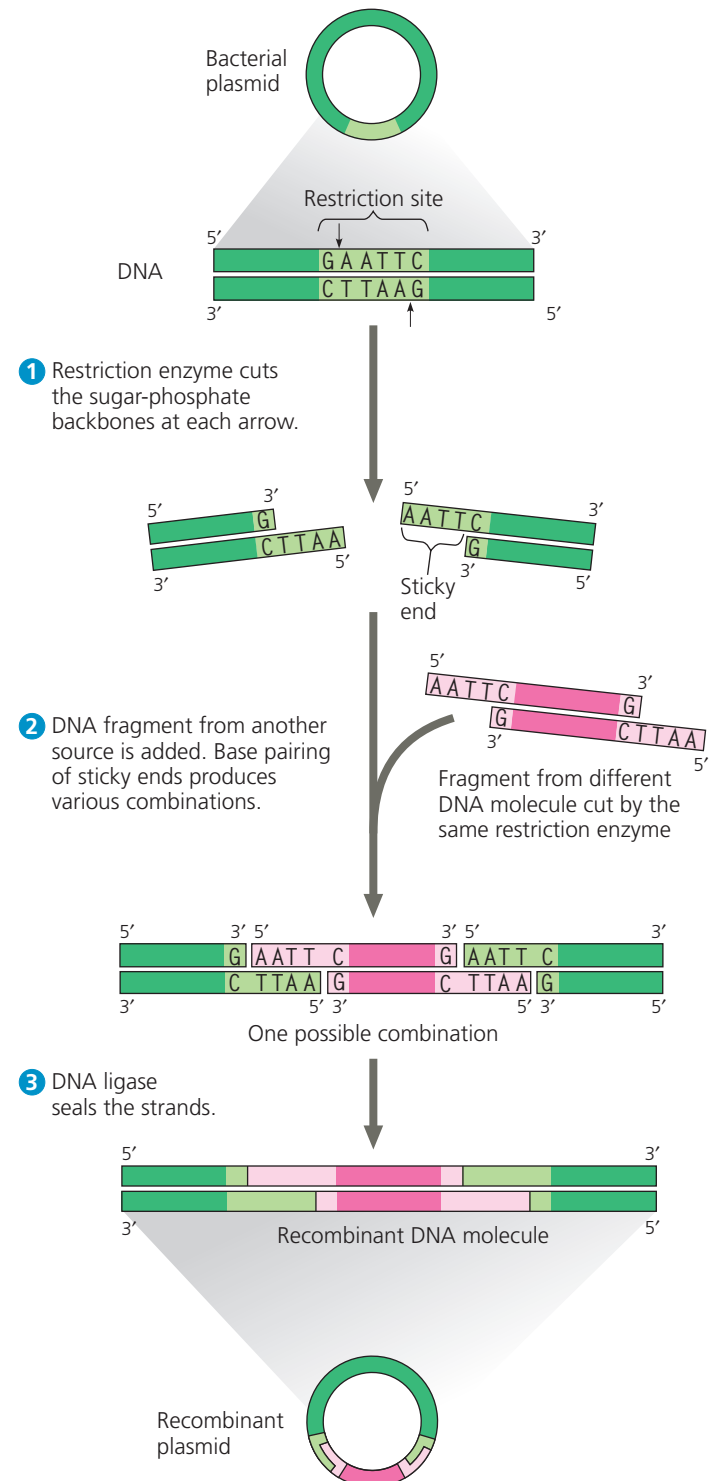
Hundreds of different restriction enzymes have been identified and isolated. Each restriction enzyme is very specific, recognizing a particular short DNA sequence, or **restriction site**, and cutting both DNA strands at precise points within this restriction site. The DNA of a bacterial cell is protected from the cell’s own restriction enzymes by the addition of methyl groups ($-\text{CH}_3$) to adenines or cytosines within the sequences recognized by the enzymes.



Animation: Restriction Enzymes

Figure 19.5 shows how restriction enzymes are used to clone a foreign DNA fragment into a bacterial plasmid. At the top is a bacterial plasmid (like the one in Figure 19.4) that has a single restriction site recognized by a particular restriction enzyme from *E. coli*. As shown in this example, most restriction sites are symmetrical. That is, the sequence of nucleotides is the same on both strands when read in the 5′ → 3′ direction. The most commonly used restriction enzymes recognize sequences containing four to eight nucleotide pairs. Because any sequence this short usually occurs (by chance)

▼ Figure 19.5 Using a restriction enzyme and DNA ligase to make a recombinant DNA plasmid. The restriction enzyme in this example (called *EcoRI*) recognizes a single six-base-pair restriction site present in this plasmid. It makes staggered cuts in the sugar-phosphate backbones, producing fragments with “sticky ends.” Foreign DNA fragments with complementary sticky ends can base-pair with the plasmid ends; the ligated product is a recombinant plasmid. (If the two plasmid sticky ends base-pair, the original nonrecombinant plasmid is reformed.)



DRAW IT ► The restriction enzyme *Hind III* recognizes the sequence 5'-AAGCTT-3', cutting between the two A's. Draw the double-stranded sequence before and after the enzyme cuts it.



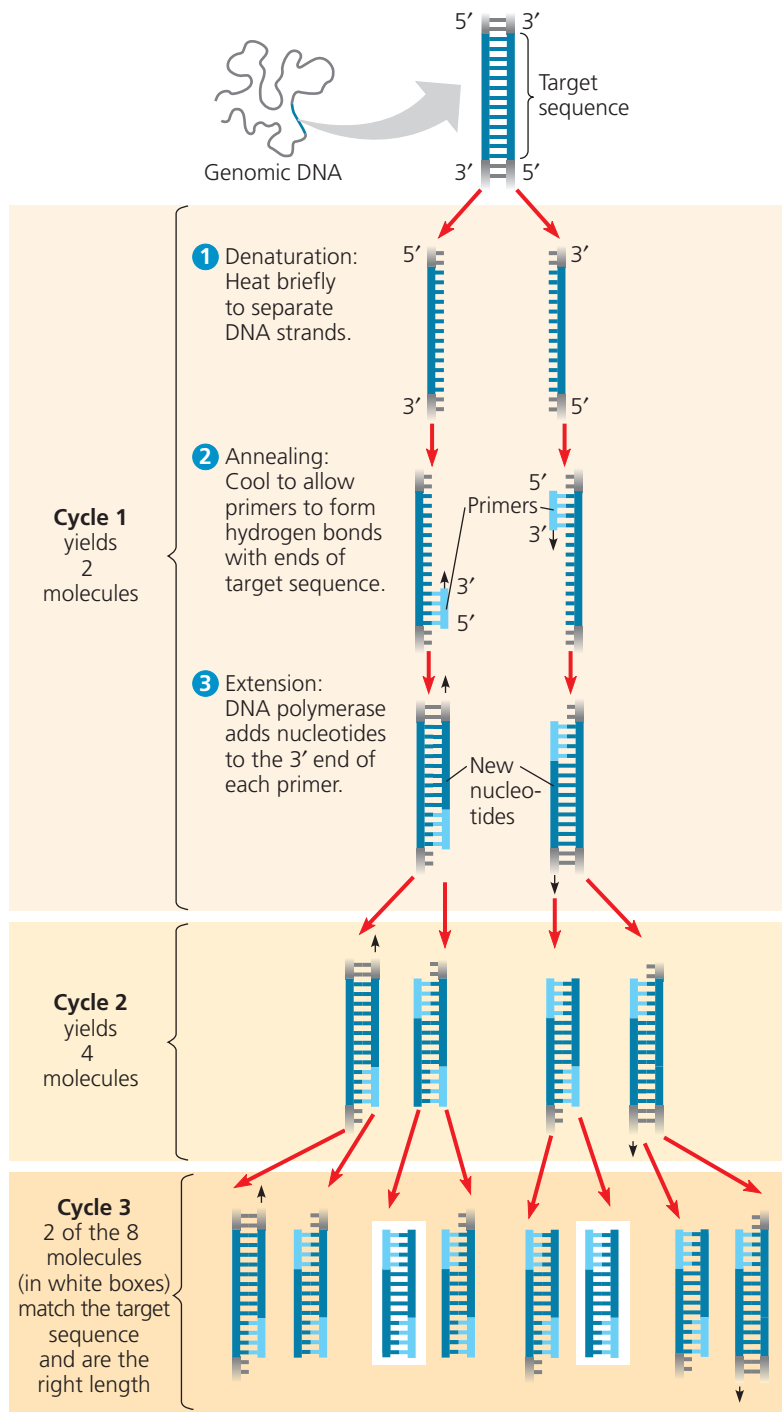
Animation: Recombinant DNA

▼ Figure 19.7

Research Method The Polymerase Chain Reaction (PCR)

Application With PCR, any specific segment—the so-called target sequence—in a DNA sample can be copied many times (amplified) within a test tube.

Technique PCR requires double-stranded DNA containing the target sequence, a heat-resistant DNA polymerase, all four nucleotides, and two 15- to 20-nucleotide single DNA strands that serve as primers. One primer is complementary to one end of the target sequence on one strand; the second primer is complementary to the other end of the sequence on the other strand.



Results After three cycles, two molecules match the target sequence exactly. After 30 more cycles, over 1 billion (10^9) molecules match the target sequence.



Animation: Copying DNA through PCR

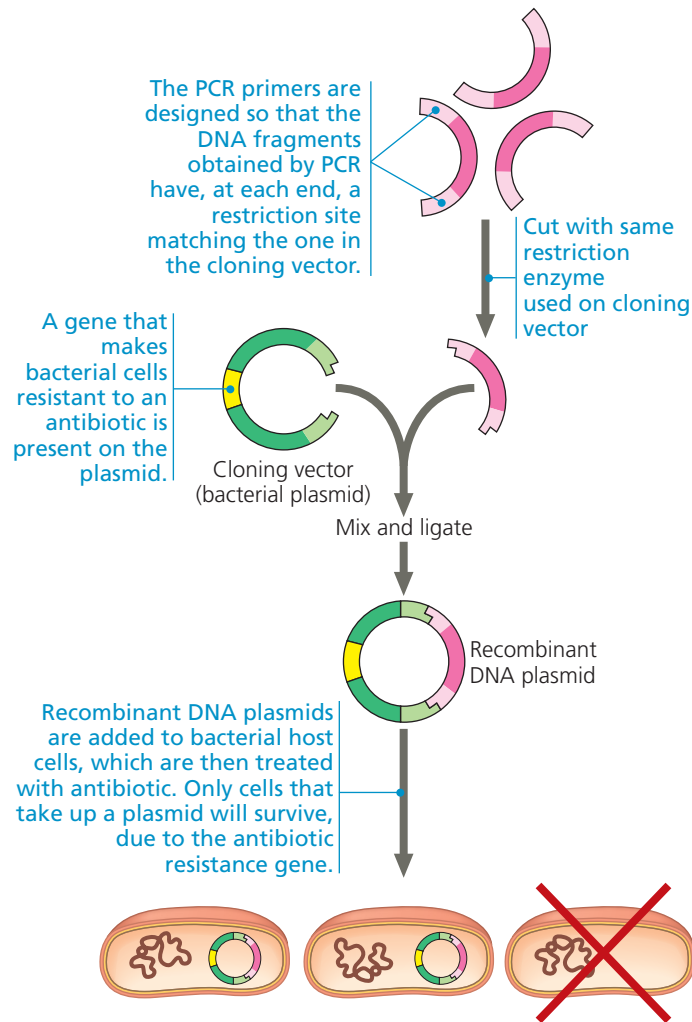
automating PCR was the discovery of an unusual heat-stable DNA polymerase called *Taq* polymerase, named after the bacterial species from which it was first isolated. This bacterial species, *Thermus aquaticus*, lives in hot springs, and the stability of its DNA polymerase at high temperatures is an evolutionary adaptation that enables the enzyme to function at temperatures up to 95°C. Today, researchers also use a DNA polymerase from the archaean species *Pyrococcus furiosus*. This enzyme, called *Pfu* polymerase, is more accurate and stable but more expensive than *Taq* polymerase.

PCR is speedy and very specific. Only a minuscule amount of DNA need be present in the starting material, and this DNA can be partially degraded, as long as there are a few copies of the complete target sequence. The key to the high specificity is the pair of primers used for each PCR amplification. The primer sequences are chosen so they hybridize *only* to sequences at opposite ends of the target segment, one on the 3' end of each strand. (For high specificity, the primers must be at least 15 or so nucleotides long.) With each successive cycle, the number of target segment molecules of the correct length doubles, so the number of molecules equals 2^n , where n is the number of cycles. After 30 or so cycles, about a billion copies of the target sequence are present!

Despite its speed and specificity, PCR amplification cannot substitute for gene cloning in cells to make large amounts of a gene. This is because occasional errors during PCR replication limit the number of good copies and the length of DNA fragments that can be copied. Instead, PCR is used to provide the specific DNA fragment for cloning. PCR primers are synthesized to include a restriction site at each end of the DNA fragment that matches the site in the cloning vector, and the fragment and vector are cut and ligated together (Figure 19.8). The resulting plasmids are sequenced so that those with error-free inserts can be selected.

Devised in 1985, PCR has had a major impact on biological research and genetic engineering. PCR has been used to amplify DNA from a wide variety of sources: a 40,000-year-old frozen woolly mammoth (see the photo on the first page of this chapter); fingerprints or tiny amounts of blood, tissue, or semen found at crime scenes; single embryonic cells for rapid prenatal diagnosis of genetic disorders (see Figure 14.19); and cells infected with viruses that are difficult to detect, such as HIV. (To test for HIV, viral genes are amplified.) We'll return to applications of PCR later.

▼ **Figure 19.8 Use of a restriction enzyme and PCR in gene cloning.** In a closer look at the process shown at the top of Figure 19.4, PCR is used to produce the DNA fragment or gene of interest that will be ligated into a cloning vector, in this case a bacterial plasmid. The ends of the fragments have the same restriction site as the cloning vector. The plasmid and the DNA fragments are cut with the same restriction enzyme and combined so the sticky ends can hybridize and be ligated together. The resulting plasmids are then introduced into bacterial host cells. The plasmid also contains an antibiotic resistance gene that allows only cells with a plasmid to survive when the antibiotic is present. Other genetic engineering techniques are used to ensure that cells with nonrecombinant plasmids can be eliminated.



Expressing Cloned Eukaryotic Genes

Once a gene has been cloned in host cells, its protein product can be expressed in large amounts for research or for practical applications, which we'll explore in Concept 19.4. Cloned genes can be expressed in either bacterial or eukaryotic cells; each option has advantages and disadvantages.

Bacterial Expression Systems

Getting a cloned eukaryotic gene to function in bacterial host cells can be difficult because certain aspects of gene

expression are different in eukaryotes and bacteria. To overcome differences in promoters and other DNA control sequences, scientists usually employ an **expression vector**, a cloning vector that contains a highly active bacterial promoter just upstream of a restriction site where the eukaryotic gene can be inserted in the correct reading frame. The bacterial host cell will recognize the promoter and proceed to express the foreign gene now linked to that promoter. Such expression vectors allow the synthesis of many eukaryotic proteins in bacterial cells.

Another problem with expressing cloned eukaryotic genes in bacteria is the presence of noncoding regions (introns) in most eukaryotic genes (see Concept 17.3). Introns can make a eukaryotic gene very long and unwieldy, and they prevent correct expression of the gene by bacterial cells, which do not have RNA-splicing machinery. This problem can be surmounted by using a form of the gene that includes only the exons. (This is called *complementary DNA*, or *cDNA*; see Figure 19.10.)

Eukaryotic DNA Cloning and Expression Systems

Molecular biologists can avoid eukaryotic-bacterial incompatibility by using eukaryotic cells such as yeasts as hosts for cloning and expressing eukaryotic genes. Yeasts, single-celled fungi, are as easy to grow as bacteria, and they have plasmids, a rarity among eukaryotes.

In addition to enabling RNA splicing, eukaryotic host cells are advantageous because many eukaryotic proteins will not function unless they are modified after translation—for example, by the addition of carbohydrate groups (glycosylation) or lipid groups. Bacterial cells cannot carry out these modifications, and if the gene product requiring such processing is from a mammal, even yeast cells may not be able to modify the protein correctly. Several cultured cell types have proved successful as host cells for this purpose, including some mammalian cell lines and an insect cell line that can be infected by a virus (called baculovirus) carrying recombinant DNA.

Besides using vectors, scientists have developed other methods for introducing recombinant DNA into eukaryotic cells. In **electroporation**, a brief electrical pulse applied to a solution containing cells creates temporary holes in their plasma membranes, through which DNA can enter. (This technique is now commonly used for bacteria as well.) Alternatively, scientists can inject DNA directly into single eukaryotic cells using microscopically thin needles. Another way to get DNA into plant cells is by using the soil bacterium *Agrobacterium tumefaciens*, as we'll discuss later. Whatever the method, if the introduced DNA is incorporated into a cell's genome by genetic recombination, then it can be expressed by the cell. Expressing different versions of genes in cells allows researchers to study protein function, a topic we'll return to in Concept 19.2.

Cross-Species Gene Expression and Evolutionary Ancestry

EVOLUTION The ability to express eukaryotic proteins in bacteria (even if the proteins aren't modified properly) is quite remarkable when we consider how different eukaryotic and bacterial cells are. In fact, examples abound of genes that are taken from one species and function perfectly well when transferred into another very different species, such as a firefly gene in a tobacco plant and a jellyfish gene in a pig (see Figure 17.7). These observations underscore the shared evolutionary ancestry of species living today.

One example involves a gene called *Pax-6*, which has been found in animals as diverse as vertebrates and fruit flies. The vertebrate *Pax-6* gene product (the PAX-6 protein) triggers a complex program of gene expression resulting in formation of the vertebrate eye, which has a single lens. Expression of the fly *Pax-6* gene leads to formation of the compound fly eye, which is quite different from the vertebrate eye. When the mouse *Pax-6* gene was cloned and introduced into a fly embryo so that it replaced the fly's own *Pax-6* gene, researchers were surprised to see that the mouse version of the gene led to formation of a compound fly eye (see Figure 50.16). Conversely, when the fly *Pax-6* gene was transferred into a vertebrate embryo—a frog, in this case—a frog eye formed. Although the genetic programs triggered in vertebrates and flies generate very different eyes, the two versions of the *Pax-6* gene can substitute for each other to trigger lens development, evidence of their evolution from a gene in a very ancient common ancestor. Because of their ancient evolutionary roots, all living organisms share the same basic mechanisms of gene expression. This commonality is the basis of many recombinant DNA techniques described in this chapter.

CONCEPT CHECK 19.1

1. **MAKE CONNECTIONS** ▶ The restriction site for an enzyme called *PvuI* is the following sequence:

5' –CGATCG–3'
3' –GCTAGC–5'

Staggered cuts are made between the T and C on each strand. What type of bonds are being cleaved? (See Concept 5.5.)

2. **DRAW IT** ▶ One strand of a DNA molecule has the following sequence:

5' –CTTGACGATCGTTACCG–3'

Draw the other strand. Will *PvuI* (see question 1) cut this molecule? If so, draw the products.

3. What are some potential difficulties in using plasmid vectors and bacterial host cells to produce large quantities of proteins from cloned eukaryotic genes?
4. **VISUAL SKILLS** ▶ Compare Figure 19.7 with Figure 16.20. How does replication of DNA ends during PCR proceed without shortening the fragments each time?

For suggested answers, see Appendix A.

CONCEPT 19.2

Biologists use DNA technology to study gene expression and function

To see how a biological system works, scientists seek to understand the functioning of the system's component parts. Analysis of when and where a gene or group of genes is expressed can provide important clues about their function.

Analyzing Gene Expression

Biologists driven to understand the assorted cell types of a multicellular organism, cancer cells, or the developing tissues of an embryo first try to discover which genes are expressed by the cells of interest. The most straightforward way to do this is usually to identify the mRNAs being made. We'll first examine techniques that look for patterns of expression of specific individual genes. Next, we'll explore ways to characterize groups of genes being expressed by cells or tissues of interest. As you will see, all of these procedures depend in some way on base pairing between complementary nucleotide sequences.

Studying the Expression of Single Genes

Suppose we have cloned a gene that we suspect plays an important role in the embryonic development of *Drosophila melanogaster* (the fruit fly). The first thing we might want to know is which embryonic cells express the gene—in other words, where in the embryo is the corresponding mRNA found? We can detect the mRNA by nucleic acid hybridization with molecules of complementary sequence that we can follow in some way. The complementary molecule, a short, single-stranded nucleic acid that can be either RNA or DNA, is called a **nucleic acid probe**. Using our cloned gene as a template, we can synthesize a probe complementary to the mRNA. For example, if part of the sequence on the mRNA were

5' ...CUC AUC ACC GGC ... 3'

then we would synthesize this single-stranded DNA probe:

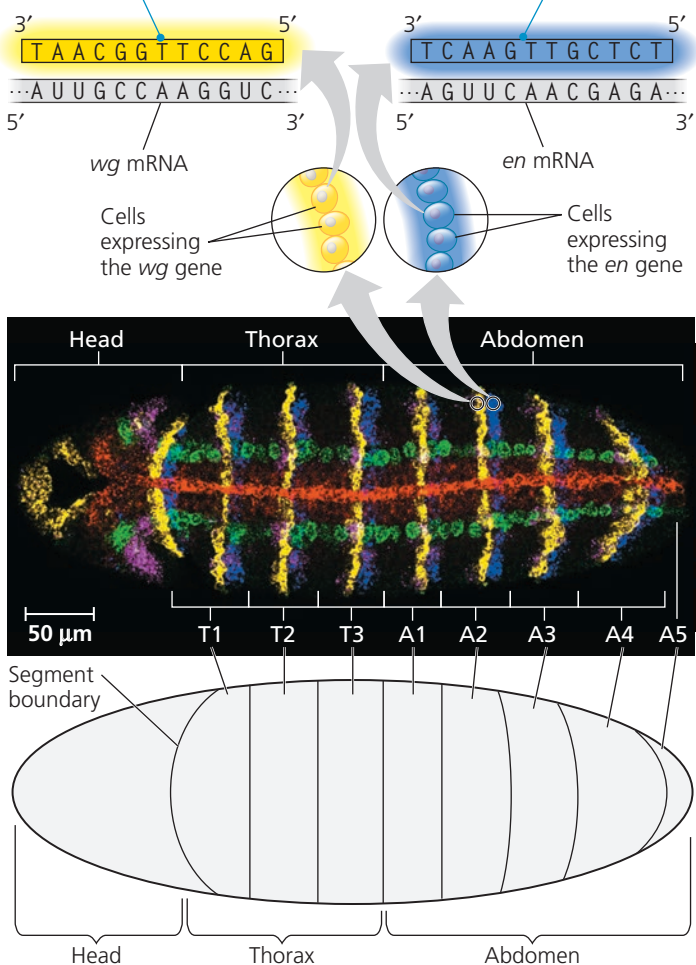
3' **GAGTAGTGGCCG** 5'

Each probe molecule is labeled during synthesis with a fluorescent tag so we can follow it. A solution containing probe molecules is applied to *Drosophila* embryos, allowing the probe to hybridize specifically with any complementary sequences on the many mRNAs in embryonic cells in which the gene is being transcribed. Because this technique allows us to see the mRNA in place (or *in situ*, in Latin) in the intact organism, this technique is called ***in situ* hybridization**. Different probes can be labeled with different fluorescent dyes, sometimes with strikingly beautiful results (Figure 19.9).

▼ **Figure 19.9 Determining where single genes are expressed by *in situ* hybridization analysis.** A *Drosophila* embryo was incubated in a solution containing DNA probes for five different mRNAs, each probe labeled with a different fluorescently colored tag. The embryo was then viewed using fluorescence microscopy; the resulting fluorescent micrograph is shown. Each color marks where a specific gene is expressed as mRNA. The arrows from the groups of yellow and blue cells above the micrograph show a magnified view of nucleic acid hybridization of the appropriately colored probe to the mRNA. Yellow cells (expressing the *wg* gene) interact with blue cells (expressing the *en* gene); their interaction helps establish the pattern in a body segment. The diagram at the bottom clarifies the eight segments visible in this view.

The yellow DNA probe hybridizes with mRNAs in cells that are expressing the *wingless* (*wg*) gene, which encodes a secreted signaling protein.

The blue DNA probe hybridizes with mRNAs in cells that are expressing the *engrailed* (*en*) gene, which encodes a transcription factor.



Other mRNA detection techniques may be preferable for comparing the amounts of a specific mRNA in several samples at the same time—for example, in different cell types or in embryos at different stages of development. One method that is widely used is called the **reverse transcriptase polymerase chain reaction**, or **RT-PCR**.

RT-PCR begins by turning sample sets of mRNAs into double-stranded DNAs with the corresponding sequences. First, the enzyme reverse transcriptase (from a virus; see

▼ **Figure 19.10 Making complementary DNA (cDNA) from eukaryotic genes.** Complementary DNA is made in a test tube using mRNA as a template for the first strand. Only one mRNA is shown here, but the final collection of cDNAs would reflect all the mRNAs present in the cell.

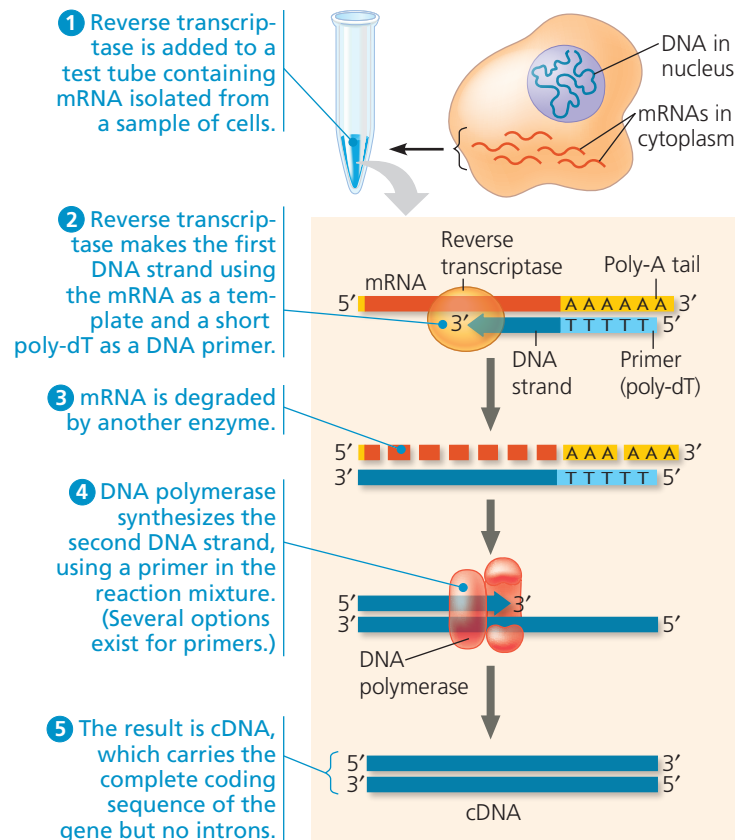


Figure 26.9) is used to synthesize a complementary DNA copy of each mRNA in the sample, called a *reverse transcript* (**Figure 19.10**). Recall that the 3' end of an mRNA has a stretch of adenine (A) nucleotides called a poly-A tail. This allows a short complementary strand of thymine deoxyribonucleotides (poly-dT) to be added and used as a primer for synthesis of this DNA strand. Following enzymatic degradation of the mRNA, a second DNA strand, complementary to the first, is synthesized by DNA polymerase. The resulting double-stranded DNA is called **complementary DNA (cDNA)**. (Made from mRNA, cDNA lacks introns and can be used for protein expression in bacteria, as mentioned earlier.) To analyze the timing of expression of the *Drosophila* gene of interest, for example, we would first isolate all the mRNAs from different stages of *Drosophila* embryos and make cDNA from each stage (**Figure 19.11**).

Next in RT-PCR is the PCR step (see Figure 19.7). As you will recall, PCR is a way of rapidly making many copies of one specific stretch of double-stranded DNA, using primers that hybridize to the opposite ends of the segment of interest. In our case, we would add primers corresponding to a segment of our *Drosophila* gene, using the cDNA from each embryonic stage as a template for PCR amplification in separate samples.

▼ Figure 19.11

Research Method RT-PCR Analysis of the Expression of Single Genes

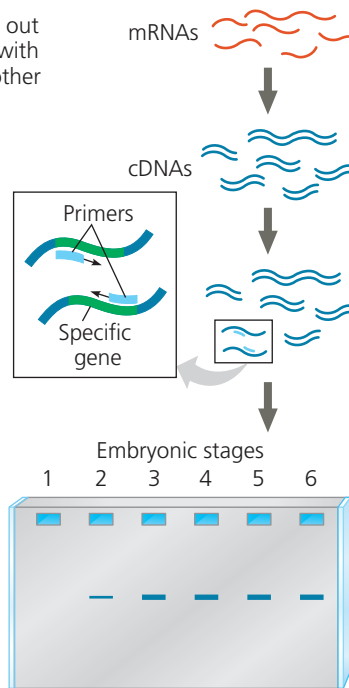
Application RT-PCR uses the enzyme reverse transcriptase (RT) in combination with PCR and gel electrophoresis. RT-PCR can be used to compare gene expression between samples—for instance, in different embryonic stages, in different tissues, or in the same type of cell under different conditions.

Technique In this example, samples containing mRNAs from six embryonic stages of *Drosophila* were analyzed for a specific mRNA as shown below. (In steps 1 and 2, the mRNA from only one stage is shown.)

1 cDNA synthesis is carried out by incubating the mRNAs with reverse transcriptase and other necessary components.

2 PCR amplification of the sample is performed using primers specific to the *Drosophila* gene of interest.

3 Gel electrophoresis will reveal amplified DNA products only in samples that contained mRNA transcribed from the specific *Drosophila* gene.



Results The mRNA for this gene first is expressed at stage 2 and continues to be expressed through stage 6. The size of the amplified fragment (shown by its position on the gel) depends on the distance between the primers that were used (not on the size of the mRNA).

When the products are analyzed on a gel, copies of the amplified region will be observed as bands only in samples that originally contained mRNA from the gene of interest. An enhancement called *quantitative RT-PCR (qRT-PCR)* uses a fluorescent dye that fluoresces only when bound to a double-stranded PCR product. The newer quantitative PCR machines can detect the light and measure the PCR product, thus avoiding the need for electrophoresis while also providing quantitative data, a distinct advantage. RT-PCR or qRT-PCR can also be carried out with mRNAs collected from different tissues at one time to discover which tissue is producing a specific mRNA.

Instructors: The **Scientific Skills Exercise** "Analyzing Quantitative and Spatial Gene Expression Data" can be assigned in MasteringBiology. Students work with data from an experiment that investigated mRNA expression using both *in situ* hybridization and quantitative RT-PCR.

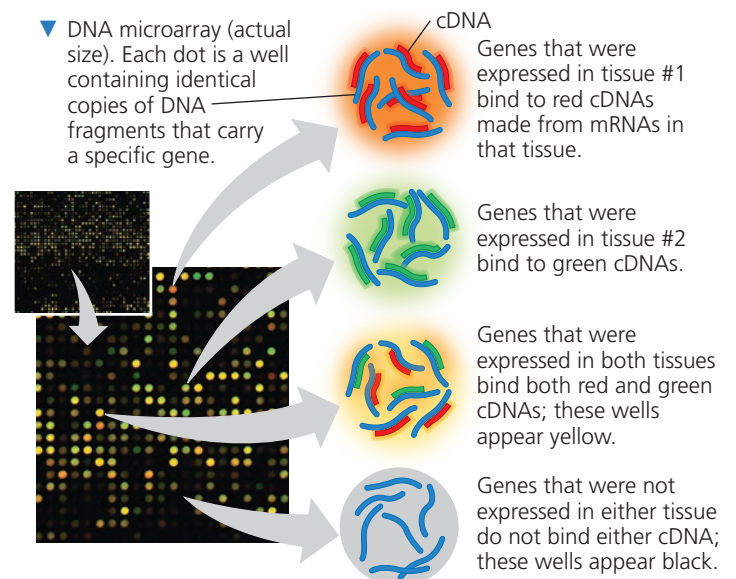
Studying the Expression of Interacting Groups of Genes

A major goal of biologists is to learn how genes act together to produce and maintain a functioning organism. Now that the genomes of a number of species have been sequenced, it is possible to study the expression of large groups of genes—the so-called *systems approach*. Researchers use what is known about the whole genome to investigate which genes are transcribed in different tissues or at different stages of development. One aim is to identify networks of gene expression across an entire genome.

Genome-wide expression studies can be carried out using **DNA microarray assays**. A DNA microarray consists of tiny amounts of a large number of single-stranded DNA fragments representing different genes fixed to a glass slide in a tightly spaced array, or grid, of dots. (The microarray is also called a *DNA chip* by analogy to a computer chip.) Ideally, these fragments represent all the genes of an organism. The mRNAs from cells under study are reverse-transcribed into cDNAs (see Figure 19.10), and a fluorescent label is added so the cDNAs can be used as probes on the microarray. Different fluorescent labels are used for different cell samples so that multiple samples can be tested in the same experiment. The resulting pattern of colored dots, shown in an actual-size microarray in **Figure 19.12**, reveals the dots to which each probe was bound and thus the genes that are expressed in the cell samples being tested. Microarray technology started taking off after several papers about it were published in 1995;

▼ Figure 19.12 Use of microarrays to analyze expression of many genes.

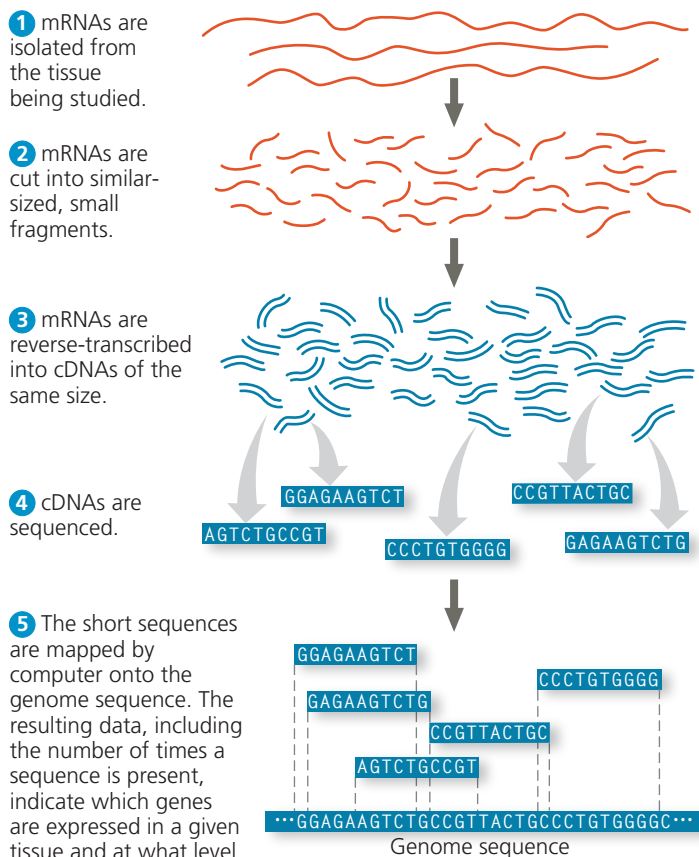
In this DNA microarray assay, researchers extracted mRNAs from two different human tissues and synthesized two sets of cDNAs, fluorescently labeled red (tissue #1) or green (tissue #2). Labeled cDNAs were hybridized with a microarray containing 5,760 human genes (about 25% of human genes), part of which is shown in the enlargement. Red indicates that the gene in that well was expressed in tissue #1, green in tissue #2, yellow in both, and black in neither. The fluorescence intensity at each spot indicates the relative expression of the gene.



since then more sophisticated applications have been developed and are in use.

Increasingly, with the advent of rapid, inexpensive DNA sequencing methods, microarray usage is decreasing: Researchers can now afford to simply sequence the cDNA samples from different tissues or different embryonic stages in order to discover which genes are expressed. This straightforward method is called **RNA sequencing**, or **RNA-seq** (pronounced “RNA-seek”), even though it is the cDNA that is actually sequenced. In RNA-seq, the mRNA (or other RNA) samples are isolated, cut into shorter, similar-sized fragments, and converted into cDNAs (**Figure 19.13**). These short cDNA stretches are sequenced, and a computer program reassembles them, either mapping them onto the genome of the species in question (when available) or simply ordering them from scratch based on overlapping sequences of multiple RNAs. RNA-seq has several advantages over microarrays. First, the procedure is not based on hybridization with a labeled probe, so it doesn’t depend on having genomic sequences in hand (although they are usually available). Second, it can measure levels of expression over a very wide range, unlike microarrays, which cannot accurately measure either very low or very high levels. Third, a careful analysis provides a wealth of information about expression of a particular gene, such as relative levels of alternatively spliced mRNAs. As the price

▼ Figure 19.13 Use of RNA sequencing (RNA-seq) to analyze expression of many genes. RNA-seq yields a wide range of information about expression of genes, including their level of expression.



of DNA sequencing plummets, RNA-seq is becoming more widely used for many applications. In most cases, however, expression of individual genes still needs to be confirmed by RT-PCR.

Scientists can now measure the expression of thousands of genes at one time. DNA technology makes such studies possible; with automation, they are easily performed on a large scale. By uncovering gene interactions and providing clues to gene function, DNA microarray assays and RNA-seq may contribute to a better understanding of diseases and suggest new diagnostic techniques or therapies. For instance, comparing patterns of gene expression in breast cancer tumors and noncancerous breast tissue has already resulted in more informed and effective treatment protocols (see Figure 18.27). Ultimately, information from these methods should provide a grander view of how ensembles of genes interact to form an organism and maintain its vital systems.

Determining Gene Function

Once they identify a gene of interest, how do scientists determine its function? A gene’s sequence can be compared with sequences in other species. If the function of a similar gene in another species is known, one might suspect that the gene product in question performs a comparable task. Data about the location and timing of gene expression may reinforce the suggested function. To obtain stronger evidence, one approach is to disable the gene and then observe the consequences in the cell or organism.

Editing Genes and Genomes

Molecular biologists have long sought techniques for altering, or editing, the genetic material of cells or organisms in a predictable way. In one such technique, called **in vitro mutagenesis**, specific mutations are introduced into a cloned gene, and the mutated gene is returned to a cell in such a way that it disables (“knocks out”) the normal cellular copies of the same gene. If the introduced mutations alter or destroy the function of the gene product, the phenotype of the mutant cell may help reveal the function of the missing normal protein. Using molecular and genetic techniques worked out in the 1980s, researchers can generate mice with any given gene disabled in order to study the role of that gene in development and in the adult. Mario Capecchi, Martin Evans, and Oliver Smithies received the Nobel Prize in 2007 for developing this technique.

Over the past 10 years, biologists have developed a powerful new technique for gene editing in living cells and organisms, called the **CRISPR-Cas9 system**, that is taking the field of genetic engineering by storm. Cas9 is a bacterial protein that helps defend bacteria against bacteriophage infections in a system worked out by Jennifer Doudna and Emmanuelle Charpentier. In bacterial cells, Cas9 acts

together with a “guide RNA” made from the CRISPR region of the bacterial system (see Figure 26.7).

Similar to the restriction enzymes described earlier, Cas9 is a nuclease that cuts double-stranded DNA molecules. However, while a given restriction enzyme recognizes only one particular DNA sequence, the Cas9 protein will cut any sequence to which it is directed. Cas9 takes its marching orders from a guide RNA molecule that it binds and uses as a homing device, cutting both strands of any DNA sequence that is exactly complementary to the guide RNA. Scientists have been able to exploit the function of Cas9 by introducing a Cas9–guide RNA complex into a cell they wish to alter (**Figure 19.14**). The guide RNA in the complex is engineered to be complementary to the “target” gene. Cas9 cuts both strands of the target DNA, and the resulting broken ends of DNA trigger a DNA repair system (similar to that shown in Figure 16.19). When there is no undamaged DNA for the enzymes of the repair system to use as a template, as shown at the bottom left of Figure 19.14, the repair enzymes rejoin the ends, sometimes introducing or removing nucleotides. If the cut is directed to a coding portion of the gene, the rejoining process often alters the DNA sequence so that the gene no longer works properly.

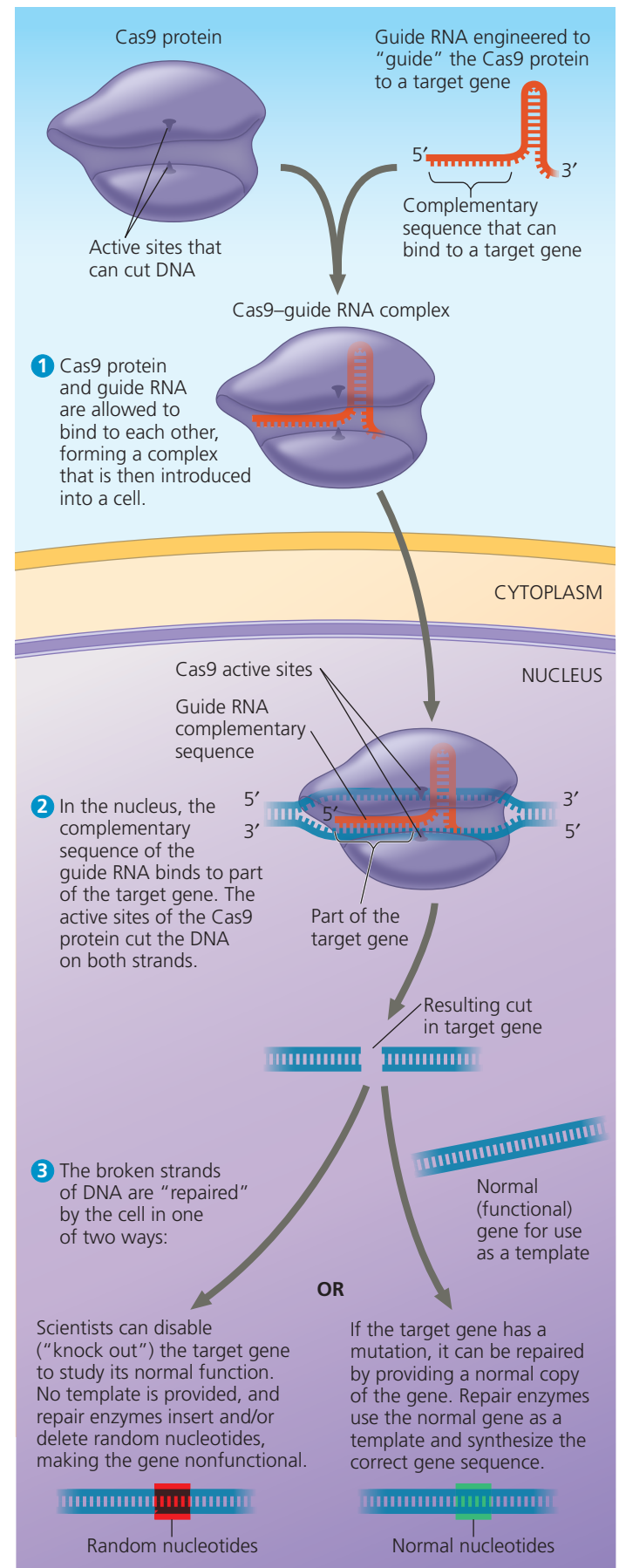
This technique is a highly effective way for researchers to knock out a given gene in order to study what that gene does, and it has already been used in many organisms, including bacteria, fish, mice, insects, human cells, and various crop plants. Researchers have also modified the technique so that CRISPR-Cas9 can be used to repair a gene that has a mutation (see bottom right of Figure 19.14). They introduce a segment from the normal (functional) gene along with the CRISPR-Cas9 system. After Cas9 cuts the target DNA, repair enzymes can use the normal DNA segment as a template to repair the target DNA at the break point. This approach is used for gene therapy, which will be discussed later in the chapter.

In another application of CRISPR-Cas9, scientists are attempting to address the global problem of insect-borne diseases by altering genes in the insect so that, for example, it cannot transmit disease. An extra twist to this approach is engineering the new allele so that it is much more highly favored for inheritance than is the wild-type allele. This is called a **gene drive** because the biased inheritance of the engineered gene during reproduction rapidly “drives” the new allele through the population.

Other Methods for Studying Gene Function

Another method for silencing expression of selected genes doesn’t alter the genome; instead, it exploits the phenomenon of **RNA interference (RNAi)**, described in Concept 18.3. This experimental approach uses synthetic double-stranded RNA molecules matching the sequence of a particular gene to trigger breakdown of the gene’s messenger RNA or to block its translation. In organisms such as the nematode

▼ **Figure 19.14** Gene editing using the CRISPR-Cas9 system.



and the fruit fly, RNAi has already proved valuable for analyzing the functions of genes on a large scale. This method is quicker than using the CRISPR-Cas9 system, but it only leads to a temporary reduction of gene expression rather than a permanent gene knockout or alteration.

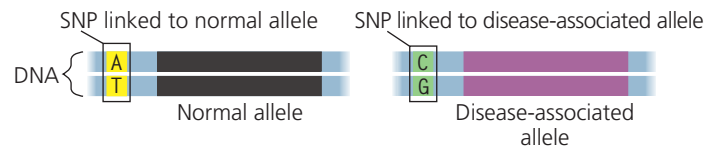
In humans, ethical considerations prohibit knocking out genes to determine their functions. An alternative approach is to analyze the genomes of large numbers of people with a certain phenotypic condition or disease, such as heart disease or diabetes, to try to find differences they all share compared with people without that condition. The assumption is that these differences may be associated with one or more malfunctioning genes, thus in a sense being naturally occurring gene knockouts. In these large-scale analyses, called **genome-wide association studies**, researchers look for *genetic markers*, DNA sequences that vary in the population. In a gene, such sequence variation is the basis of different alleles, as we have seen for sickle-cell disease (see Figure 17.26). And just like the coding sequences of genes, noncoding DNA at a specific locus on a chromosome may exhibit small nucleotide differences among individuals. Variations in coding or noncoding DNA sequences among a population are called polymorphisms (from the Greek for “many forms”).

Among the most useful genetic markers in tracking down genes that contribute to diseases and disorders are single base-pair variations in the genomes of the human population. A single base-pair site where variation is found in at least 1% of the population is called a **single nucleotide polymorphism (SNP)**, pronounced “snip”). A few million SNPs occur in the human genome, about once in 100–300 base pairs of both coding and noncoding DNA sequences. To find SNPs in large numbers of people, it isn’t necessary to sequence their DNA; SNPs can be detected by very sensitive microarray assays, RNA-seq, or PCR.

Once a SNP is identified that is found in all affected people, researchers focus on that region and sequence it. In nearly all cases, the SNP itself does not contribute directly to the disease in question by altering the encoded protein; in fact, most SNPs are in noncoding regions. Instead, if the SNP and a disease-associated allele are close enough to be genetically linked, scientists can take advantage of the fact that crossing over between the marker and the gene is very unlikely during gamete formation. Therefore, the marker and gene will almost always be inherited together, even though the marker is not part of the gene (**Figure 19.15**). SNPs have been found that correlate with diabetes, heart disease, and several types of cancer, and the search is on for genes that might be involved.

The experimental approaches you have learned about thus far focused on working with molecules, mainly DNA and proteins. In a parallel line of inquiry, biologists have been

▼ **Figure 19.15 Single nucleotide polymorphisms (SNPs) as genetic markers for disease-associated alleles.** This diagram depicts the same region of the genome from two groups of individuals, one group having a particular disease or condition with a genetic basis. Unaffected people have an A/T pair at a given SNP locus, while affected people have a C/G pair there. Once the allele is confirmed as being associated with the disease in question, the SNP that varies in this way can be used as a marker for the disease-associated allele.



developing powerful techniques for cloning whole multicellular organisms. One aim of this work is to obtain special types of cells, called stem cells, that can give rise to all types of tissues. Being able to manipulate stem cells would allow scientists to use the DNA-based methods previously discussed to alter stem cells for the treatment of diseases. Methods involving the cloning of organisms and production of stem cells are the subject of the next section.

CONCEPT CHECK 19.2

1. Describe the role of complementary base pairing during RT-PCR, DNA microarray analysis, RNA sequencing, and CRISPR-Cas9 editing.
2. **VISUAL SKILLS** ► Consider the microarray in Figure 19.12. If a sample from normal tissue is labeled with a green fluorescent dye and a sample from cancerous tissue is labeled red, what color spots would represent genes you would be interested in if you were studying cancer? Explain.

For suggested answers, see Appendix A.

CONCEPT 19.3

Cloned organisms and stem cells are useful for basic research and other applications

Along with advances in DNA technology, scientists have been developing and refining methods for cloning whole multicellular organisms from single cells. In this context, cloning produces one or more organisms that are genetically identical to the “parent” that donated the single cell. This is often called *organismal cloning* to differentiate it from gene cloning and, more significantly, from cell cloning—the division of an asexually reproducing cell such as a bacterium into a group of genetically identical cells. (The common theme is that the product is genetically identical to the parent. In fact, the word *clone* comes from the Greek *klon*, meaning “twig.”) The current interest in organismal cloning arises primarily from its ability to generate stem cells. A **stem cell** is a relatively

unspecialized cell that can both reproduce itself indefinitely and, under appropriate conditions, differentiate into specialized cells of one or more types. Stem cells have great potential for regenerating damaged tissues.

The cloning of plants and animals was first attempted over 50 years ago in experiments designed to answer basic biological questions. For example, researchers wondered if all the cells of an organism have the same genes or whether cells lose genes during the process of differentiation (see Concept 18.4). One way to answer this question is to see whether a differentiated cell can generate a whole organism—in other words, whether cloning an organism is possible. Let's discuss these early experiments before we consider more recent progress in organismal cloning and procedures for producing stem cells.

Cloning Plants: Single-Cell Cultures

The successful cloning of whole plants from single differentiated cells was accomplished during the 1950s by F. C. Steward and his students at Cornell University, who worked with carrot plants. They found that differentiated cells taken from the root (the carrot) and incubated in culture medium could grow into normal adult plants, each genetically identical to the parent plant. These results showed that differentiation does not necessarily involve irreversible changes in the DNA. In plants, mature cells can “dedifferentiate” and then give rise to all the specialized cell types of the organism. Any cell with this potential is said to be **totipotent**.

Plant cloning is used extensively in agriculture. For plants such as orchids, cloning is the only commercially practical means of reproducing plants. In other cases, cloning has been used to reproduce a plant with valuable characteristics, such as resistance to plant pathogens. In fact, you yourself may be a plant cloner: If you have ever grown a new plant from a cutting, you have practiced cloning!

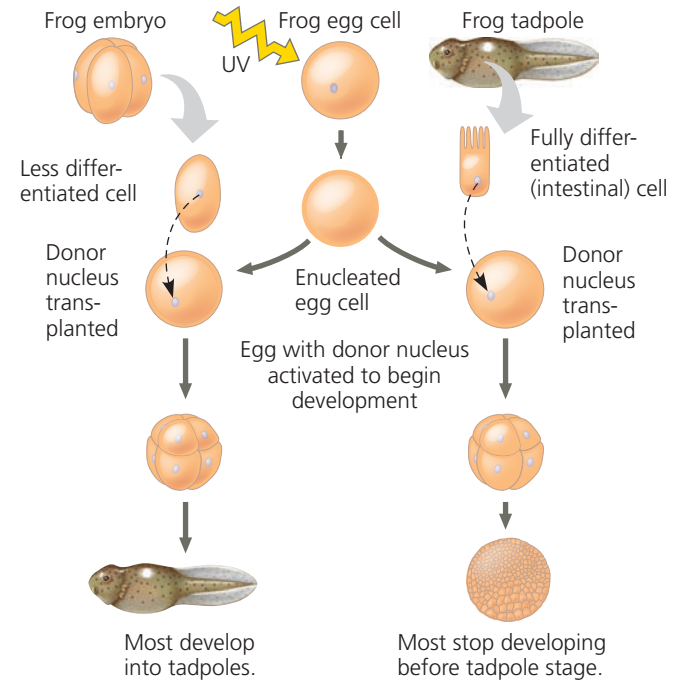
Cloning Animals: Nuclear Transplantation

Differentiated cells from animals generally do not divide in culture, much less develop into the multiple cell types of a new organism. Therefore, early researchers had to use a different approach to answer the question of whether differentiated animal cells are totipotent. Their approach was to remove the nucleus of an egg (creating an *enucleated* egg) and replace it with the nucleus of a differentiated cell, a procedure called *nuclear transplantation*, now more commonly called *somatic cell nuclear transfer*. If the nucleus from the differentiated donor cell retains its full genetic capability, then it should be able to direct development of the recipient cell into all the tissues and organs of an organism. Such experiments were conducted on one species of frog (*Rana pipiens*) by Robert Briggs and Thomas King in the 1950s and on another frog species (*Xenopus laevis*) by John Gurdon in the 1970s (Figure 19.16). These researchers transplanted a nucleus from an embryonic or tadpole cell into an enucleated egg of the same species. In

Figure 19.16

Inquiry Can the nucleus from a differentiated animal cell direct development of an organism?

Experiment John Gurdon and colleagues at Oxford University, in England, destroyed the nuclei of frog (*Xenopus laevis*) eggs by exposing the eggs to ultraviolet light. They then transplanted nuclei from cells of frog embryos and tadpoles into the enucleated eggs.



Results When the transplanted nuclei came from an early embryo, the cells of which are relatively undifferentiated, most of the recipient eggs developed into tadpoles. But when the nuclei came from the fully differentiated intestinal cells of a tadpole, fewer than 2% of the eggs developed into normal tadpoles, and most of the embryos stopped developing at a much earlier stage.

Conclusion The nucleus from a differentiated frog cell can direct development of a tadpole. However, its ability to do so decreases as the donor cell becomes more differentiated, presumably because of changes in the nucleus.

Data from J. B. Gurdon et al., The developmental capacity of nuclei transplanted from keratinized cells of adult frogs, *Journal of Embryology and Experimental Morphology* 34:93–112 (1975).

WHAT IF? > If each cell in a four-cell embryo were already so specialized that it was not totipotent, what results would you predict for the experiment on the left side of the figure?



HHMI Video: Somatic Cell Nuclear Transfer



Gurdon's experiments, the transplanted nucleus was often able to support normal development of the egg into a tadpole. However, he found that the potential of a transplanted nucleus to direct normal development was inversely related to the age of the donor: The older the donor nucleus, the lower the percentage of normal tadpoles (see Figure 19.16).

From these results, Gurdon concluded that something in the nucleus *does* change as animal cells differentiate. In

► **Figure 19.17**
Reproductive cloning of a mammal by nuclear transfer.

Dolly, shown here as a lamb, has a very different appearance from her surrogate mother, standing beside her.



frogs and most other animals, nuclear potential tends to be restricted more and more as embryonic development and cell differentiation progress. These were foundational experiments that ultimately led to stem cell technology, and Gurdon received the 2012 Nobel Prize in Medicine for this work.

Reproductive Cloning of Mammals

In addition to cloning frogs, researchers were able to clone mammals using early embryonic cells as a source of donor nuclei. Until about 20 years ago, though, it was not known whether a nucleus from a fully differentiated cell could be reprogrammed successfully to act as a donor nucleus. In 1997, researchers in Scotland announced the birth of Dolly, a lamb cloned from an adult sheep by nuclear transfer from a differentiated mammary gland cell (**Figure 19.17**). Using a technique related to that in 19.16, the researchers implanted early embryos into surrogate mothers. Out of several hundred embryos, one successfully completed normal development, and Dolly was born, a genetic clone of the nucleus donor. At the age of 6, Dolly suffered complications from a lung infection often seen in sheep kept indoors and was euthanized. Another cloned sheep from the same experiment developed an unusual lung disease. This led to speculation that this sheep's cells were in some way not quite as healthy as those of a normal sheep, possibly reflecting incomplete reprogramming of the original transplanted nucleus. Reprogramming involves epigenetic changes that lead to changes in chromatin structure (see Concept 18.2), to be discussed shortly.

Since that time, researchers have cloned numerous other mammals, including mice, cats, cows, horses, pigs, dogs, and monkeys. In most cases, their goal has been the production of new individuals; this is known as *reproductive cloning*. We have already learned a lot from such experiments. For example, cloned animals of the same species do *not* always look or behave identically. In a herd of cows cloned from the same line of cultured cells, certain cows are dominant in behavior and others are more submissive. Another example of non-identity in clones is the first cloned cat, named CC for Carbon Copy (**Figure 19.18**). She has a calico coat, like her single

▼ **Figure 19.18** CC (“Carbon Copy”), the first cloned cat (**right**), and her single parent. Rainbow (left) donated the nucleus in a cloning procedure that resulted in CC. However, the two cats are not identical: Rainbow is a classic calico cat with orange patches on her fur and has a “reserved personality,” while CC has a gray and white coat and is more playful.



female parent, but the color and pattern are different because of random X chromosome inactivation, which is a normal occurrence during embryonic development (see Figure 15.8). And identical human twins, which are naturally occurring “clones,” are always slightly different. Clearly, environmental influences and random phenomena play a significant role during development.

Faulty Gene Regulation in Cloned Animals Due to Epigenetic Differences

In most nuclear transplantation studies thus far, only a small percentage of cloned embryos develop normally to birth. And like Dolly, many cloned animals exhibit defects. Cloned mice, for instance, are prone to obesity, pneumonia, liver failure, and premature death. Scientists assert that even cloned animals that appear normal are likely to have subtle defects.

Researchers have uncovered some reasons for the low efficiency of cloning and the high incidence of abnormalities. In the nuclei of fully differentiated cells, a small subset of genes is turned on and expression of the rest of the genes is repressed. This regulation often is the result of epigenetic changes in chromatin, such as acetylation of histones or methylation of DNA (see Figure 18.7). During the nuclear transfer procedure, many of these changes must be reversed in the later-stage nucleus from a donor animal for genes to be expressed or repressed appropriately in early stages of development. Researchers have found that the DNA in cells from cloned embryos, like that of differentiated cells, often has more methyl groups than does the DNA in equivalent cells from normal embryos of the same species. This finding suggests that the reprogramming of donor nuclei requires more accurate

and complete chromatin restructuring than occurs during cloning procedures. Because DNA methylation helps regulate gene expression, misplaced or extra methyl groups in the DNA of donor nuclei may interfere with the pattern of gene expression necessary for normal embryonic development. In fact, the success of a cloning attempt may depend in large part on whether or not the chromatin in the donor nucleus can be artificially modified to resemble that of a newly fertilized egg.

Stem Cells of Animals

Progress in cloning mammalian embryos, including primates, has heightened speculation about the cloning of humans, which has not yet been achieved past very early embryonic stages. The main reason researchers have been trying to clone human embryos is not for reproduction, but for the production of stem cells to treat human diseases. Recall that a stem cell is a relatively unspecialized cell that can both reproduce itself indefinitely and, under appropriate conditions, differentiate into specialized cells of one or more types (**Figure 19.19**). Thus, stem cells are able to both replenish their own population and generate cells that travel down specific differentiation pathways.



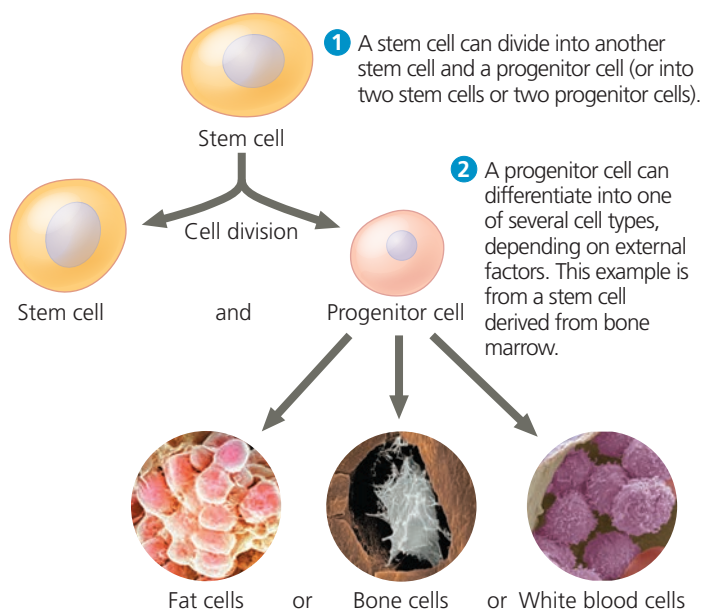
HHMI Animation: Somatic Cell Nuclear Transfer



Embryonic and Adult Stem Cells

Many early animal embryos contain stem cells capable of giving rise to differentiated cells of any type. Stem cells can be isolated from early embryos at a stage called the blastula stage or its human equivalent, the blastocyst stage. In culture, these *embryonic stem (ES) cells* reproduce indefinitely, and depending on culture conditions, they can be made to differentiate

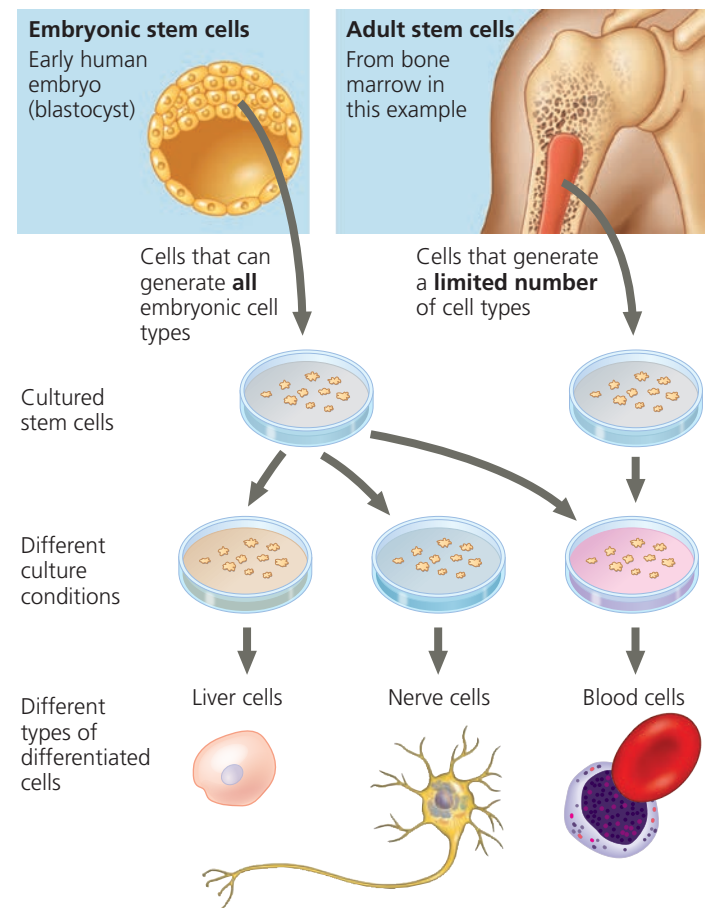
▼ **Figure 19.19** How stem cells maintain their own population and generate differentiated cells.



HHMI Video: Cultured Human Embryonic Stem Cells



▼ **Figure 19.20** Working with stem cells. Animal stem cells, which can be isolated from early embryos or adult tissues and grown in culture, are self-perpetuating, relatively undifferentiated cells. Embryonic stem cells are easier to grow than adult stem cells and can theoretically give rise to *all* types of cells in an organism. The range of cell types that can arise from adult stem cells is not yet fully understood.



HHMI Animation: Creating Embryonic Stem Cell Lines



into a wide variety of specialized cells (**Figure 19.20**), including even eggs and sperm.

The adult body also has stem cells, which serve to replace nonreproducing specialized cells as needed. In contrast to ES cells, *adult stem cells* are not able to give rise to all cell types in the organism, though they can generate several defined types. For example, one of the several types of stem cells in bone marrow can generate all the different kinds of blood cells (see Figure 19.20), and another type of bone marrow stem cell can differentiate into bone, cartilage, fat, muscle, and the linings of blood vessels. To the surprise of many, the adult brain has been found to contain stem cells that continue to produce certain kinds of nerve cells there. Researchers have also reported finding stem cells in skin, hair, eyes, and dental pulp. Although adult animals have only tiny numbers of stem cells, scientists are learning to identify and isolate these cells from various tissues and, in some cases, to grow them in culture. With the right culture conditions (for instance, the addition of specific growth factors), cultured stem cells from adult animals have been made to differentiate

into various defined types of specialized cells, although none are as versatile as ES cells.

Research with embryonic or adult stem cells is a source of valuable data about differentiation and has enormous potential for medical applications. The ultimate aim is to supply cells for the repair of damaged or diseased organs: for example, insulin-producing pancreatic cells for people with type 1 diabetes or certain kinds of brain cells for people with Parkinson's disease or Huntington's disease. Adult stem cells from bone marrow have long been used in bone marrow transplants as a source of immune system cells in patients whose own immune systems are nonfunctional because of genetic disorders or radiation treatments for cancer.

The developmental potential of adult stem cells is limited to certain tissues. ES cells hold more promise than adult stem cells for most medical applications because ES cells are **pluripotent**, capable of differentiating into many different cell types. In 2013, a research group reported that they had established ES cell lines from human blastocysts produced by transferring a nucleus from a differentiated cell into an enucleated egg. Prior to that report, cells were obtained only from embryos donated by patients undergoing infertility treatments or from long-term cell cultures originally established with cells isolated from donated embryos, an issue that prompts ethical and political discussions. Although the techniques for cloning early human embryos are still being optimized, they represent a potential new source for ES cells that may be less controversial. Furthermore, with a donor nucleus from a person with a particular disease, researchers should be able to produce ES cells that match the patient and are thus not rejected by his or her immune system when used for treatment. When the main aim of cloning is to produce ES cells to treat disease, the process is called *therapeutic cloning*. Although most people believe that reproductive cloning of humans is unethical, opinions vary about the morality of therapeutic cloning.

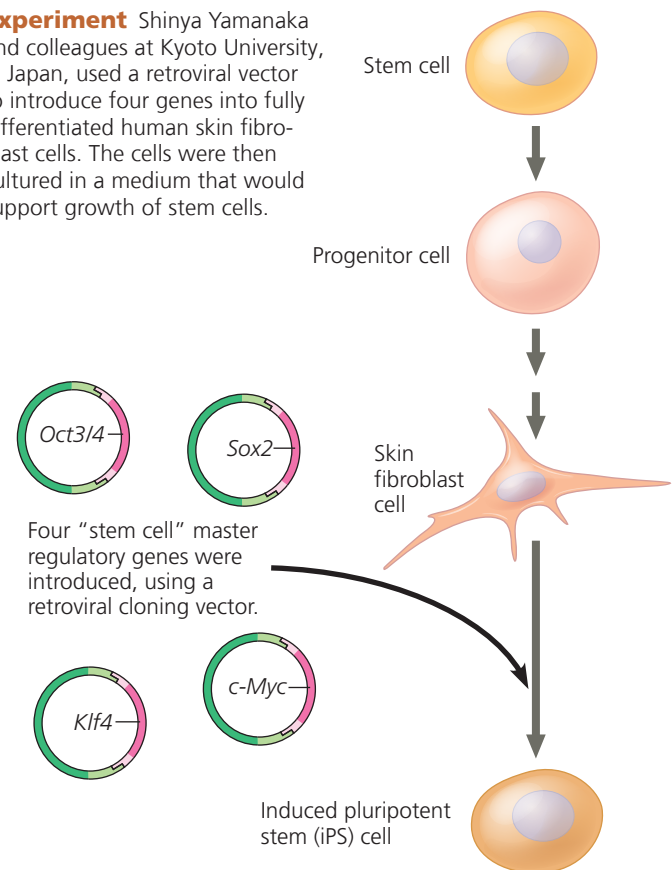
Induced Pluripotent Stem (iPS) Cells

Resolving the debate now seems less urgent because researchers have learned to turn back the clock in fully differentiated cells, reprogramming them to act like ES cells. The accomplishment of this feat, which posed formidable obstacles, was announced in 2007, first by labs using mouse skin cells and then by additional groups using cells from human skin and other organs or tissues. In all these cases, researchers transformed the differentiated cells into a type of ES cell by using a retrovirus (a specific class of virus; see Table 26.1) to introduce extra, cloned copies of four "stem cell" master regulatory genes. The "deprogrammed" cells are known as *induced pluripotent stem (iPS)* cells because, in using this fairly simple laboratory technique to return them to their undifferentiated state, pluripotency has been restored. The experiments that first transformed human differentiated cells into iPS cells are described in **Figure 19.21**. Shinya Yamanaka received the

▼ **Figure 19.21**

Inquiry Can a fully differentiated human cell be "deprogrammed" to become a stem cell?

Experiment Shinya Yamanaka and colleagues at Kyoto University, in Japan, used a retroviral vector to introduce four genes into fully differentiated human skin fibroblast cells. The cells were then cultured in a medium that would support growth of stem cells.



Results Two weeks later, the cells resembled embryonic stem cells in appearance and were actively dividing. Their gene expression patterns, gene methylation patterns, and other characteristics were also consistent with those of embryonic stem cells. The iPS cells were able to differentiate into heart muscle cells, as well as other cell types.

Conclusion The four genes induced differentiated skin cells to become pluripotent stem cells, with characteristics of embryonic stem cells.

Data from K. Takahashi et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, *Cell* 131:861–872 (2007).

WHAT IF? ► Patients with diseases such as heart disease or Alzheimer's could have their own skin cells reprogrammed to become iPS cells. Once procedures have been developed for converting iPS cells into heart or nervous system cells, the patients' own iPS cells might be used to treat their disease. When organs are transplanted from a donor to a diseased recipient, the recipient's immune system may reject the transplant, a dangerous condition. Would using iPS cells be expected to carry the same risk? Why or why not? Given that these cells are actively dividing, undifferentiated cells, what risks might this procedure carry?

2012 Nobel Prize in Medicine for this work, shared with John Gurdon, whose work you read about in Figure 19.16.

By many criteria, iPS cells can perform most of the functions of ES cells, but there are some differences in gene expression and other cellular functions, such as cell division. At least until these differences are fully understood, the study

of ES cells will continue to make important contributions to the development of stem cell therapies. (In fact, it is likely that ES cells will always be a focus of basic research as well.) In the meantime, work is proceeding using the iPS cells that have been experimentally produced.

There are two major potential uses for human iPS cells. First, cells from patients suffering from diseases have been reprogrammed to become iPS cells, which act as model cells for studying the disease and potential treatments. Human iPS cell lines have already been developed from individuals with type 1 diabetes, Parkinson's disease, Huntington's disease, Down syndrome, and many other diseases. Second, in the field of regenerative medicine, a patient's own cells could be reprogrammed into iPS cells and then used to replace nonfunctional tissues, such as insulin-producing cells of the pancreas. In fact, in 2014 two research groups described successful methods for growing insulin-producing cells from both iPS cells and ES cells. Before these cells can be used in patients, however, researchers will have to develop a way to ensure the cells are not destroyed by the patients' immune system (the original cause of type 1 diabetes, in which the immune system malfunctions).

In another surprising development, researchers have been able to identify genes that can directly reprogram a differentiated cell into another type of differentiated cell without it passing through a pluripotent state. In the first reported example, one type of cell in the pancreas was transformed into another type. However, the two types of cells do not need to be very closely related: Another research group has been able to directly reprogram a skin fibroblast into a nerve cell. Development techniques that direct iPS cells or even fully differentiated cells to become specific cell types for regenerative medicine is an area of intense research, one that has already seen some success. The iPS cells created in this way could eventually provide tailor-made "replacement" cells for patients without using any human eggs or embryos, thus circumventing most ethical objections.

CONCEPT CHECK 19.3

1. Based on current knowledge, how would you explain the difference in the percentage of tadpoles that developed from the two kinds of donor nuclei in Figure 19.16?
2. A few companies in China and South Korea provide the service of cloning dogs, using cells from their clients' pets to provide nuclei in procedures like that in Figure 19.17. Should their clients expect the clone to look identical to their original pet? Why or why not? What ethical questions does this bring up?
3. **MAKE CONNECTIONS** > Based on what you know about muscle differentiation (see Figure 18.18) and genetic engineering, propose the first experiment you might try if you wanted to direct an embryonic stem cell or iPS cell to develop into a muscle cell.

For suggested answers, see Appendix A.

CONCEPT 19.4

The practical applications of DNA-based biotechnology affect our lives in many ways

DNA technology is in the news almost every day. Most often, the topic is a new and promising application in medicine, but this is just one of numerous fields benefiting from DNA technology and genetic engineering.

Medical Applications

One important use of DNA technology is the identification of human genes whose mutation plays a role in genetic diseases. These discoveries may lead to ways of diagnosing, treating, and even preventing such conditions. DNA technology is also contributing to our understanding of "nongenetic" diseases, from arthritis to AIDS, since a person's genes influence susceptibility to these diseases. Furthermore, diseases of all sorts involve changes in gene expression within the affected cells and often within the patient's immune system. By using RNA-seq and DNA microarray assays (see Figures 19.12 and 19.13) or other techniques to compare gene expression in healthy and diseased tissues, researchers are finding genes that are turned on or off in particular diseases. These genes and their products are potential targets for prevention or therapy.

Diagnosis and Treatment of Diseases

A new chapter in the diagnosis of infectious diseases has been opened by DNA technology, in particular the use of PCR and labeled nucleic acid probes to track down pathogens. For example, because the sequence of the RNA genome of HIV is known, RT-PCR can be used to amplify, and thus detect, HIV RNA in blood or tissue samples (see Figure 19.11). RT-PCR is often the best way to detect an otherwise elusive infective agent.

Medical scientists can now diagnose hundreds of human genetic disorders by using PCR with primers that target the genes associated with these disorders. The amplified DNA product is then sequenced to reveal the presence or absence of the disease-causing mutation. Among the genes for human diseases that have been identified are those for sickle-cell disease, hemophilia, cystic fibrosis, Huntington's disease, and Duchenne muscular dystrophy. Individuals with such diseases can often be identified before the onset of symptoms, even before birth (see Figure 14.19). PCR can also be used to identify symptomless carriers of potentially harmful recessive alleles.

As you learned earlier, genome-wide association studies have pinpointed SNPs (single nucleotide polymorphisms) that are linked to disease-associated alleles (see Figure 19.15).

Individuals can be tested by PCR and sequencing for a SNP that is correlated with the abnormal allele. The presence of particular SNPs is correlated with increased risk for conditions such as heart disease, Alzheimer's disease, and some types of cancer. Companies that offer individual genetic testing for risk factors like these are looking for previously identified, linked SNPs. It may be helpful for individuals to learn about their health risks, with the understanding that such genetic tests merely reflect correlations and do not make predictions.

The techniques described in this chapter have also prompted improvements in disease treatments. By analyzing the expression of many genes in breast cancer patients, researchers have been able to refine their understanding of the different subtypes of breast cancer (see Figure 18.27). Knowing the expression levels of particular genes can help physicians determine the likelihood that the cancer will recur, thus helping them design an appropriate treatment. Given that some low-risk patients have a 96% survival rate over a ten-year period with no treatment, gene expression analysis allows doctors and patients access to valuable information when they are considering treatment options.

Many envision a future of “personalized medicine” where each person's genetic profile can inform them about diseases or conditions for which they are especially at risk and help them make treatment choices. As we will discuss later in the chapter, a *genetic profile* is currently taken to mean a set of genetic markers such as SNPs. Ultimately, however, it will likely mean the complete DNA sequence of an individual—once sequencing becomes inexpensive enough. Our ability to sequence a person's genome rapidly and inexpensively is advancing faster than our development of appropriate treatments for the conditions we are characterizing. Still, the identification of genes involved in these conditions provides us with good targets for therapeutic interventions.

Human Gene Therapy and Gene Editing

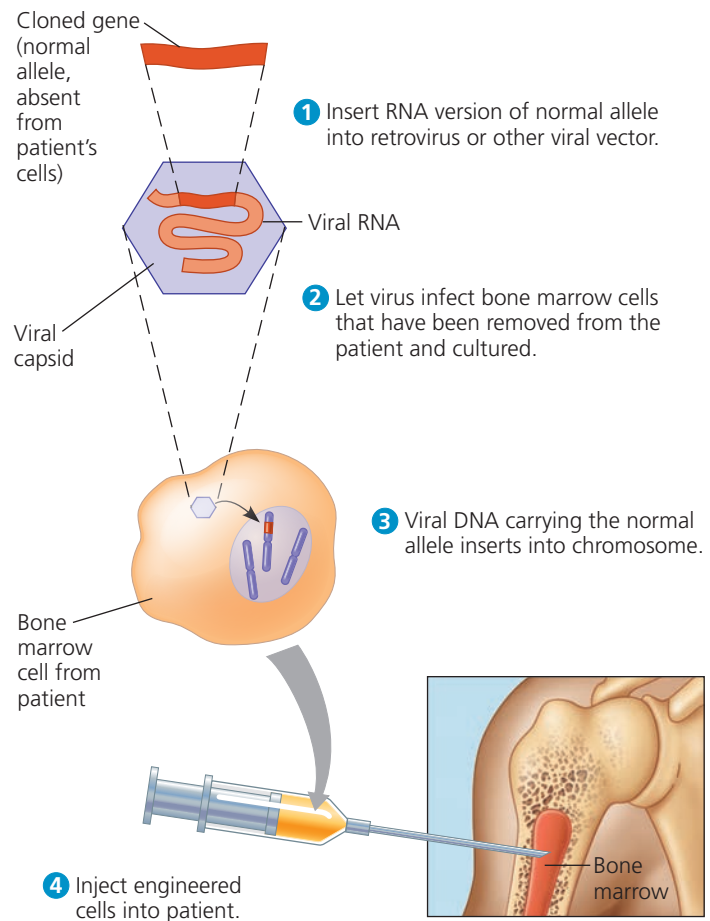
Gene therapy—the introduction of genes into an afflicted individual for therapeutic purposes—holds great potential for treating the relatively small number of disorders traceable to a single defective gene. The aim of this approach is to insert a normal allele of the defective gene into the somatic cells of the tissue affected by the disorder.

For gene therapy of somatic cells to be permanent, the cells that have the normal allele must be cells that multiply throughout the patient's life. Bone marrow cells, which include the stem cells that give rise to all the cells of the blood and immune system, are prime candidates.

Figure 19.22 outlines one procedure for gene therapy in an individual whose bone marrow cells do not produce a vital

▼ Figure 19.22 Gene therapy using a retroviral vector.

A retrovirus that has been rendered harmless is used as a vector in this procedure, which exploits the ability of a retrovirus to insert a DNA transcript of its RNA genome into the chromosomal DNA of its host cell (see Figure 26.9). If the foreign gene carried by the retroviral vector is expressed, the cell and its descendants will possess the gene product. Cells that reproduce throughout life, such as bone marrow cells, are ideal candidates for gene therapy.



enzyme because of a single defective gene. One type of severe combined immunodeficiency (SCID) is caused by this kind of defect. If the treatment is successful, the patient's bone marrow cells will begin producing the missing protein, and the patient may be cured.

The procedure shown in Figure 19.22 was used in gene therapy trials for SCID in France in 2000. In that trial, ten young children with SCID were treated by the same procedure. Nine of these patients showed significant, definitive improvement after two years, the first indisputable success of gene therapy. However, three of the patients subsequently developed leukemia, a type of blood cell cancer, and one of them died. Researchers have concluded it is likely that the insertion of the retroviral vector occurred near a gene that triggers the proliferation of blood cells. Using a viral vector that does not come from a retrovirus, clinical researchers have treated at least three other genetic diseases somewhat

successfully with gene therapy: a type of progressive blindness (see Concept 50.3), a degenerative disease of the nervous system, and a blood disorder involving the β -globin gene.

Gene therapy raises many technical issues. For example, how can the activity of the transferred gene be controlled so that cells make appropriate amounts of the gene product at the right time and in the right place? How can we be sure that the insertion of the therapeutic gene does not harm some other necessary cell function? As more is learned about DNA control elements and gene interactions, researchers may be able to answer such questions.

A more direct approach that avoids the complications of using a viral vector in gene therapy is made possible by gene editing, especially given the development of the CRISPR-Cas9 system described earlier. In this approach the existing defective gene is edited to correct the mutation. As shown in Figure 19.14, the CRISPR-Cas9 system is capable of doing this.

In 2014, a group of researchers reported correcting a genetic defect in mice using CRISPR-Cas9 technology. The lab mice had been genetically engineered to have a mutation in a gene encoding a liver enzyme that metabolizes the amino acid tyrosine, mimicking a fatal genetic disorder in humans called tyrosinemia. A guide RNA molecule complementary to the mutated region of the gene was introduced into the mouse along with the Cas9 protein and a segment of DNA from the same region of the normal gene for use as a template. Subsequent analysis indicated that the faulty gene had been corrected in enough of the liver cells that the amount of functional enzyme made was sufficient to alleviate the disease symptoms. There are still hurdles to overcome before this approach can be used in clinical trials in humans, but the CRISPR technology is sparking widespread excitement among researchers and physicians alike.

In addition to technical challenges, gene therapy and gene editing provoke ethical questions. Some critics believe that tampering with human genes in any way is immoral or unethical. Other observers see no fundamental difference between the transplantation of genes into somatic cells and the transplantation of organs. You might wonder whether scientists are considering engineering human germ-line cells in the hope of correcting a defect in future generations. Such genetic engineering is now routinely done in laboratory mice, and, in fact, conditions that would allow genetic engineering of human embryos have been worked out.

The development of the CRISPR-Cas9 system has engendered much debate about the ethics of gene editing, related to applications both potential and real. In March of 2015, an editorial was published by leading scientists working with CRISPR-Cas9 calling for the research community to “strongly discourage” any experimental work on human eggs or embryos. A month later, however, scientists in China reported using CRISPR-Cas9 technology to edit a gene in human embryos. (They used “nonviable” fertilized eggs—zygotes—that would not develop all the way but could form

blastocysts.) The researchers were attempting to edit the β -thalassemia gene, mutations in which cause a blood disease of the same name. They injected 86 zygotes, only four of which showed the gene to be edited properly. In many of the other embryos, there were effects on genes other than the β -thalassemia gene—at a much higher level than had been seen in mouse embryos or human cell lines. This study highlighted problems with the technique, at least in human embryos, and at the same time accelerated concern about ethical considerations. Under what circumstances, if any, should we alter the genomes of human germ lines? Would this inevitably lead to the practice of eugenics, a deliberate effort to control the genetic makeup of human populations? While we may not have to resolve these questions immediately, considering them is imperative because they will likely come to the fore at some point in the near future.

Pharmaceutical Products

The pharmaceutical industry derives significant benefit from advances in DNA technology and genetic research, applying them to the development of useful drugs to treat diseases. Pharmaceutical products are synthesized using methods of either organic chemistry or biotechnology, depending on the nature of the product.

Synthesis of Small Molecules for Use as Drugs

Determining the sequence and structure of proteins crucial for tumor cell survival has led to the identification of small molecules that combat certain cancers by blocking the function of these proteins. One drug, imatinib (trade name Gleevec), is a small molecule that inhibits one tyrosine kinase (see Figure 9.8). The overexpression of this kinase, resulting from a chromosomal translocation, is instrumental in causing chronic myelogenous leukemia (CML; see Figure 15.16). Patients in the early stages of CML who are treated with imatinib have exhibited nearly complete, sustained remission from the cancer. Drugs that work in a similar way have also been used with success to treat a few types of lung and breast cancers. This approach is feasible only for cancers for which the molecular basis is fairly well understood.



HHMI Video: Gleevec



In many cases of such drug-treated tumors, though, cells later arise that are resistant to the new drug. In one study, the whole genome of the tumor cells was sequenced both before and after the appearance of drug resistance. Comparison of the sequences showed genetic changes that allowed the tumor cells to “get around” the drug-inhibited protein. Here, we can see that cancer cells demonstrate the principles of evolution: Certain tumor cells have a random mutation that allows them to survive in the presence of a particular drug, and as a consequence of natural selection in the presence of the drug, these are the cells that survive and reproduce.

Protein Production in Cell Cultures Pharmaceutical products that are proteins are commonly synthesized on a large scale using cell cultures. You learned earlier in the chapter about DNA cloning and gene expression systems for producing large quantities of a chosen protein that is present naturally in only minute amounts. The host cells used in such expression systems can even be engineered to secrete a protein as it is made, thereby simplifying the task of purifying it by traditional biochemical methods.

Among the first pharmaceutical products manufactured in this way were human insulin and human growth hormone (HGH). Some 2 million people with diabetes in the United States depend on insulin treatment to control their disease. Human growth hormone has been a boon to children born with a form of dwarfism caused by inadequate amounts of HGH, as well as helping AIDS patients gain weight. Another important pharmaceutical product produced by genetic engineering is tissue plasminogen activator (TPA). If administered shortly after a heart attack, TPA helps dissolve blood clots and reduces the risk of subsequent heart attacks.

Protein Production by “Pharm” Animals In some cases, instead of using cell systems to produce large quantities of protein products, pharmaceutical scientists can use whole animals. They can introduce a gene (or other DNA) from an animal into the genome of another individual, often of a different species. This individual is then called a **transgenic** animal. To do this, they first remove eggs from a female of the recipient species and fertilize them *in vitro*. Meanwhile, they have cloned the desired gene from the donor organism. They then inject the cloned DNA directly into the nuclei of the fertilized eggs. Some of the cells integrate the foreign DNA, the *transgene*, into their genome and are able to express the foreign gene. The engineered embryos that arise from these zygotes are then surgically implanted in a surrogate mother. If an embryo develops successfully, the result is a transgenic animal that expresses its new, “foreign” gene.

Assuming that the introduced gene encodes a protein desired in large quantities, these transgenic animals can act as pharmaceutical “factories.” For example, a transgene for a human blood protein such as antithrombin, which prevents blood clots, can be inserted into the genome of a goat in such a way that the transgene’s product is secreted in the animal’s milk (**Figure 19.23**). The protein is then purified from the milk (which is easier than purification from a cell culture). Such proteins must be tested to ensure that they (or contaminants from the farm animals) will not cause allergic reactions or other adverse effects in patients who receive them.

Forensic Evidence and Genetic Profiles

In violent crimes, body fluids or small pieces of tissue may be left at the scene or on the clothes or other possessions of the victim or assailant. If enough blood, semen, or tissue

▼ **Figure 19.23 Goats as “pharm” animals.** This transgenic goat carries a gene for a human blood protein, antithrombin, which she secretes in her milk. Patients with a rare hereditary disorder in which this protein is lacking suffer from formation of blood clots in their blood vessels. Easily purified from the goat’s milk, the protein is used to prevent blood clots in these patients during surgery or childbirth.



is available, forensic laboratories can determine the blood type or tissue type by using antibodies to detect specific cell-surface proteins. However, such tests require fairly fresh samples in relatively large amounts. Also, because many people have the same blood or tissue type, this approach can only exclude a suspect; it cannot provide strong evidence of guilt.

DNA testing, on the other hand, can identify the guilty individual with a high degree of certainty because the DNA sequence of every person is unique (except for identical twins). Genetic markers that vary in the population can be analyzed for a given person to determine that individual’s unique set of genetic markers, or **genetic profile**. (This term is preferred over “DNA fingerprint” by forensic scientists, who want to emphasize the heritable aspect of these markers rather than the fact that they produce a pattern on a gel that, like a fingerprint, is visually recognizable.) The FBI started applying DNA technology to forensics in 1988, using a method involving gel electrophoresis and nucleic acid hybridization to detect similarities and differences in DNA samples. This method required much smaller samples of blood or tissue than earlier methods—only about 1,000 cells.



Animation: Genetic Profiles

Today, forensic scientists use an even more sensitive method that takes advantage of variations in length of genetic markers called **short tandem repeats (STRs)**. These are tandemly repeated units of two- to five-nucleotide sequences in specific regions of the genome. The number of repeats present in these regions is highly variable from person to person (polymorphic), and even for a single individual, the two alleles of an STR may differ from each other. For example, one individual may have the sequence ACAT repeated

30 times at one genome locus and 15 times at the same locus on the other homolog, whereas another individual may have 18 repeats at this locus on each homolog. (These two genotypes can be expressed by the two repeat numbers: 30,15 and 18,18.) PCR is used to amplify particular STRs, using sets of primers that are labeled with different-colored fluorescent tags; the length of the region, and thus the number of repeats, can then be determined by electrophoresis. The PCR step allows use of this method even when the DNA is in poor condition or available only in minute quantities: A tissue sample containing as few as 20 cells can be sufficient.

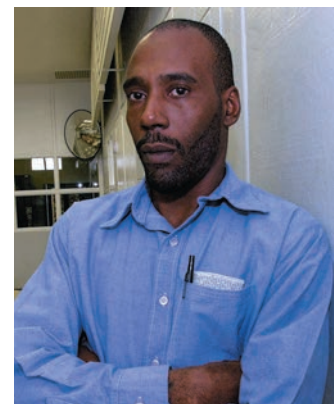
In a murder case, for example, this method can be used to compare DNA samples from the suspect, the victim, and a small amount of blood found at the crime scene. The forensic scientist tests only a few selected portions of the DNA—usually 13 STR markers. However, even this small set of markers can provide a forensically useful genetic profile because the probability that two people (who are not identical twins) would have exactly the same set of STR markers is vanishingly small. The Innocence Project, a nonprofit organization dedicated to overturning wrongful convictions, uses STR analysis of archived samples from crime scenes to revisit old cases. As of 2016, more than 340 innocent people have been released from prison as a result of forensic and legal work by this group (Figure 19.24).

Genetic profiles can also be useful for other purposes. A comparison of the DNA of a mother, her child, and the purported father can conclusively settle a question of paternity. Sometimes paternity is of historical interest: Genetic profiles provided strong evidence that Thomas Jefferson or one of his close male relatives fathered at least one of the children of his slave Sally Hemings. Genetic profiles can also identify victims of mass casualties. The largest such effort occurred after the attack on the World Trade Center in 2001; more than 10,000 samples of victims' remains were compared with DNA samples from personal items, such as toothbrushes, provided by families. Ultimately, forensic scientists succeeded in identifying almost 3,000 victims using these methods.

Just how reliable is a genetic profile? The greater the number of markers examined in a DNA sample, the more likely it is that the profile is unique to one individual. In forensic cases using STR analysis with 13 markers, the probability of two people having identical DNA profiles is somewhere between one chance in 10 billion and one in several trillion. (For comparison, the world's population is between 7 and 8 billion.) The exact probability depends on the frequency of those markers in the general population. Information on how common various markers are in different ethnic groups is critical because these marker frequencies may vary considerably among ethnic groups and between a particular ethnic group and the population as a whole. With the increasing availability of frequency data, forensic scientists can make extremely accurate statistical calculations. Thus, despite problems that

▼ **Figure 19.24 STR analysis used to release an innocent man from prison.**

(a) In 1984, Earl Washington was convicted and sentenced to death for the 1982 rape and murder of Rebecca Williams. His sentence was commuted to life in prison in 1993 due to new doubts about the evidence. In 2000, STR analysis by forensic scientists associated with the Innocence Project showed conclusively that he was innocent. This photo shows Washington just before his release in 2001, after 17 years in prison.



Source of sample	STR marker 1	STR marker 2	STR marker 3
Semen on victim	17,19	13,16	12,12
Earl Washington	16,18	14,15	11,12
Kenneth Tinsley	17,19	13,16	12,12

(b) In STR analysis, selected STR markers in a DNA sample are amplified by PCR, and the PCR products are separated by electrophoresis. The procedure reveals how many repeats are present for each STR locus in the sample. An individual has two alleles per STR locus, each with a certain number of repeats. This table shows the number of repeats for three STR markers in three samples: from semen found on the victim, from Washington, and from another man (Kenneth Tinsley), who was in prison because of an unrelated conviction. These and other STR data (not shown) exonerated Washington and led Tinsley to plead guilty to the murder.

can still arise from insufficient data, human error, or flawed evidence, genetic profiles are now accepted as compelling evidence by legal experts and scientists alike.

Environmental Cleanup

Increasingly, the diverse abilities of certain microorganisms to transform chemicals are being exploited for environmental cleanup. If the growth needs of such microorganisms make them unsuitable for direct use, scientists can now transfer the genes for their valuable metabolic capabilities into other microorganisms, which can then be used to treat environmental problems. For example, many bacteria can extract heavy metals, such as copper, lead, and nickel, from their environments and incorporate the metals into compounds such as copper sulfate or lead sulfate, which are readily recoverable. Genetically engineered microorganisms may become important in both mining (especially as ore reserves are depleted) and cleaning up highly toxic mining wastes. Biotechnologists are also trying to engineer microorganisms that can degrade chlorinated hydrocarbons and other harmful compounds.

These microorganisms could be used in wastewater treatment plants or by manufacturers before the compounds are ever released into the environment.

Agricultural Applications

Scientists are working to learn more about the genomes of agriculturally important plants and animals. For a number of years, they have been using DNA technology in an effort to improve agricultural productivity. The selective breeding of both livestock (animal husbandry) and crops has exploited naturally occurring mutations and genetic recombination for thousands of years.

As we described earlier, DNA technology enables scientists to produce transgenic animals, which speeds up the selective breeding process. The goals of creating a transgenic animal are often the same as the goals of traditional breeding—for instance, to make a sheep with better quality wool, a pig with leaner meat, or a cow that will mature in a shorter time. Scientists might, for example, identify and clone a gene that causes the development of larger muscles (muscles make up most of the meat we eat) in one breed of cattle and transfer it to other cattle or even to sheep. However, health problems are not uncommon among farm animals carrying genes from other species, and modification of the animal's own genes using the CRISPR-Cas9 system will likely emerge as a more useful technique. Animal health and welfare are important issues to consider when genetically altering animals.

Agricultural scientists have already endowed a number of crop plants with genes for desirable traits, such as delayed ripening and resistance to spoilage, disease, and drought. Modifications can also add value to food crops, giving them a longer shelf life or improved flavor or nutritional value. For many plant species, a single tissue cell grown in culture can give rise to an adult plant. Thus, genetic manipulations can be performed on an ordinary somatic cell and the cell then used to generate a plant with new traits.

Genetic engineering is rapidly replacing traditional plant-breeding programs, especially for useful traits, such as herbicide or pest resistance, determined by one or a few genes. Crops engineered with a bacterial gene making the plants resistant to an herbicide can grow while weeds are destroyed, and genetically engineered crops that can resist destructive insects reduce the need for chemical insecticides. In India, the insertion of a salinity resistance gene from a coastal mangrove plant into the genomes of several rice varieties has resulted in rice plants that can grow in water three times as salty as seawater. The research foundation that carried out this feat of genetic engineering estimates that one-third of all irrigated land has high salinity owing to overirrigation and intensive use of chemical fertilizers, representing a serious threat to the food supply. Thus, salinity-resistant crop plants would be enormously valuable worldwide.

Safety and Ethical Questions Raised by DNA Technology

Early concerns about potential dangers associated with recombinant DNA technology focused on the possibility that hazardous new pathogens might be created. What might happen, for instance, if in a research study cancer cell genes were transferred into bacteria or viruses? To guard against such rogue microorganisms, scientists developed a set of guidelines that were adopted as formal government regulations in the United States and some other countries. One safety measure is a set of strict laboratory procedures designed to prevent engineered microorganisms from infecting researchers or accidentally leaving the laboratory. In addition, strains of microorganisms to be used in recombinant DNA experiments are genetically crippled to ensure that they cannot survive outside the laboratory. Finally, certain obviously dangerous experiments have been banned.

Today, most public concern about possible hazards centers not on recombinant microorganisms but on **genetically modified organisms (GMOs)** used as food. A GMO is a transgenic organism, one that has acquired by artificial means one or more genes from another species or even from another variety of the same species. Some salmon, for example, have been genetically modified by addition of a more active salmon growth hormone gene. However, the majority of the GMOs that contribute to our food supply are not animals, but crop plants.

GM crops are widespread in the United States, Argentina, and Brazil; together, these countries account for over 80% of the world's acreage devoted to such crops. In the United States, most corn, soybean, and canola crops are genetically modified, and a recent law requires labeling of GM products. The same foods are an ongoing subject of controversy in Europe, where the GM revolution has been met with strong opposition. Many Europeans are concerned about the safety of GM foods and the possible environmental consequences of growing GM plants. Although a small number of GM crops have been grown on European soil, the European Union established a comprehensive legal framework regarding GMOs in 2015. Among other regulations, individual member states may ban either the growing or importing of GM crops, which must be clearly labeled. The high degree of consumer distrust in Europe makes the future of GM crops there uncertain.

Advocates of a cautious approach toward GM crops fear that transgenic plants might pass their new genes to close relatives in nearby wild areas. We know that lawn and crop grasses, for example, commonly exchange genes with wild relatives via pollen transfer. If crop plants carrying genes for resistance to herbicides, diseases, or insect pests pollinated wild ones, the offspring might become “super weeds” that are very difficult to control. Another worry involves possible risks to human health from GM foods. Some people fear that the protein products of transgenes might lead to allergic

reactions. Although there is some evidence that this could happen, advocates claim that these proteins could be tested in advance to avoid producing ones that cause allergic reactions. (For further discussion of plant biotechnology and GM crops, see Concept 38.3.)

Today, governments and regulatory agencies throughout the world are grappling with how to facilitate the use of biotechnology in agriculture, industry, and medicine while ensuring that new products and procedures are safe. In the United States, such applications of biotechnology must be evaluated for potential risks by various regulatory agencies, including the Food and Drug Administration, the Environmental Protection Agency, the National Institutes of Health, and the Department of Agriculture. Meanwhile, these same agencies and the public must consider the ethical implications of biotechnology.



ABC News Video: Genetically Altered Salmon

Advances in biotechnology have allowed us to obtain complete genome sequences for humans and many other species, providing a vast treasure trove of information about genes. We can ask how certain genes differ from species to species, as well as how genes and, ultimately, entire genomes have evolved. (These are the subjects of Chapter 20.) At the same time, the increasing speed and falling cost of sequencing the genomes of individuals are raising significant ethical questions. Who should have the right to examine someone else's genetic information? How should that information be used?

Should a person's genome be a factor in determining eligibility for a job or insurance? Ethical considerations, as well as concerns about potential environmental and health hazards, will likely slow some applications of biotechnology.

There is always a danger that too much regulation will stifle basic research and its potential benefits. On the other hand, genetic engineering—especially gene editing with the CRISPR-Cas system—enables us to profoundly and rapidly alter species that have been evolving for millennia. A good example is the potential use of a gene drive that would eliminate the ability of mosquito species to carry diseases or even eradicate certain mosquito species. There would probably be health benefits to this approach, at least initially, but unforeseen problems could easily arise. Given the tremendous power of DNA technology, we must proceed with humility and caution.



Interview with David Suzuki: Exploring ethical issues related to DNA technology

CONCEPT CHECK 19.4

1. What is the advantage of using stem cells for gene therapy or gene editing?
2. List at least three different properties that have been acquired by crop plants via genetic engineering.
3. **WHAT IF? >** As the investigator of a murder case, how can you use DNA technology to identify the guilty? Discuss the genetic basis of this technology.

For suggested answers, see Appendix A.

19 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 19.1

DNA sequencing and DNA cloning are valuable tools for genetic engineering and biological inquiry (pp. 448–455)



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax

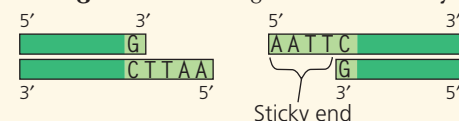
- **Nucleic acid hybridization**, the base pairing of one strand of a nucleic acid to the complementary sequence on a strand from another nucleic acid molecule, is widely used in **DNA technology**.
- **DNA sequencing** can be carried out using the dideoxy chain termination method in automated sequencing machines.
- Next-generation (high-throughput) techniques for sequencing DNA are based on sequencing by synthesis: DNA polymerase is used to synthesize a stretch of DNA from a single-stranded template, and the order in which nucleotides are added reveals the sequence. Third-generation sequencing methods, including nanopore technology, sequence long DNA molecules one at a



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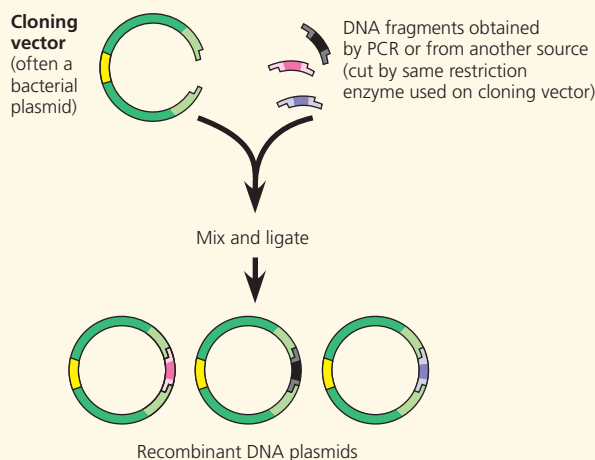
time by distinguishing the nucleotide bases as they pass through a pore in a membrane.

- **Gene cloning** (or **DNA cloning**) produces multiple copies of a gene (or DNA segment) that can be used to manipulate and analyze DNA and to produce useful new products or organisms with beneficial traits.
- In **genetic engineering**, bacterial **restriction enzymes** are used to cut DNA molecules within short, specific nucleotide sequences (**restriction sites**), yielding a set of double-stranded **restriction fragments** with single-stranded **sticky ends**:



- The sticky ends on restriction fragments from one DNA source can base-pair with complementary sticky ends on fragments from other DNA molecules. Sealing the base-paired fragments with DNA ligase produces **recombinant DNA molecules**.
- DNA restriction fragments of different lengths can be separated by **gel electrophoresis**.

- The **polymerase chain reaction (PCR)** can produce many copies of (amplify) a specific target segment of DNA *in vitro*, using primers that bracket the desired sequence and a heat-resistant DNA polymerase.
- To clone a eukaryotic gene:



Recombinant plasmids are returned to host cells, each of which divides to form a clone of cells.

- Several technical difficulties hinder the expression of cloned eukaryotic genes in bacterial host cells. The use of cultured eukaryotic cells as host cells, coupled with appropriate **expression vectors**, helps avoid these problems.

? Describe how the process of gene cloning results in a cell clone containing a recombinant plasmid.

CONCEPT 19.2

Biologists use DNA technology to study gene expression and function (pp. 455–460)

- Several techniques use hybridization of a **nucleic acid probe** to detect the presence of specific mRNAs.
- **In situ hybridization** and **RT-PCR** can detect the presence of a given mRNA in a tissue or an RNA sample, respectively.
- **DNA microarrays** are used to identify sets of genes co-expressed by a group of cells. Increasingly, instead, **RNA sequencing (RNA-seq)** is used to sequence the **cDNAs** corresponding to RNAs from the cells.
- For a gene of unknown function, experimental inactivation of the gene (a gene knockout) and observation of the resulting phenotypic effects can provide clues to its function. The **CRISPR-Cas9 system** allows researchers to edit genes in living cells in a specific, desired way. The new alleles can be altered so that they are inherited in a biased way through a population (**gene drive**). In humans, **genome-wide association studies** identify and use **single nucleotide polymorphisms (SNPs)** as genetic markers for alleles that are associated with particular conditions.

? What useful information is obtained by detecting expression of specific genes?

CONCEPT 19.3

Cloned organisms and stem cells are useful for basic research and other applications (pp. 460–465)

- The question of whether all the cells in an organism have the same genome prompted the first attempts at organismal cloning.
- Single differentiated cells from plants are often **totipotent**: capable of generating all the tissues of a complete new plant.

- Transplantation of the nucleus from a differentiated animal cell into an enucleated egg can sometimes give rise to a new animal.
- Certain embryonic **stem cells** (ES cells) from animal embryos and particular adult stem cells from adult tissues can reproduce and differentiate both in the lab and in the organism, offering the potential for medical use. ES cells are **pluripotent** but difficult to acquire. Induced pluripotent stem (iPS) cells resemble ES cells in their capacity to differentiate; they can be generated by reprogramming differentiated cells. iPS cells hold promise for medical research and regenerative medicine.

? Describe how, using mice, a researcher could carry out (1) organismal cloning, (2) production of ES cells, and (3) generation of iPS cells, focusing on how the cells are reprogrammed. (The procedures are basically the same in humans and mice.)

CONCEPT 19.4

The practical applications of DNA-based biotechnology affect our lives in many ways (pp. 465–471)

- DNA technology, including the analysis of genetic markers such as SNPs, is increasingly being used in the diagnosis of genetic and other diseases and offers potential for better treatment of genetic disorders or even permanent cures through **gene therapy**, or gene editing with the CRISPR-Cas9 system. It also enables more informed cancer therapies. DNA technology is used with cell cultures in the large-scale production of protein hormones and other proteins with therapeutic uses. Some therapeutic proteins are being produced in **transgenic** “pharm” animals.
- Analysis of genetic markers such as **short tandem repeats (STRs)** in DNA isolated from tissue or body fluids found at crime scenes leads to a **genetic profile**. Use of genetic profiles can provide definitive evidence that a suspect is innocent or strong evidence of guilt. Such analysis is also useful in parenthood disputes and in identifying the remains of crime victims.
- Genetically engineered microorganisms can be used to extract minerals from the environment or degrade various types of toxic waste materials.
- The aims of developing transgenic plants and animals are to improve agricultural productivity and food quality.
- The potential benefits of genetic engineering must be carefully weighed against the potential for harm to humans or the environment.

? What factors affect whether a given genetic disease would be a good candidate for successful gene therapy?

TEST YOUR UNDERSTANDING

Multiple-choice Self-Quiz questions 1–8 can be found in the Study Area in MasteringBiology.

9. **MAKE CONNECTIONS** Imagine you want to study one of the human crystallins, proteins present in the lens of the eye (see Figure 1.8). To obtain a sufficient amount of the protein of interest, you decide to clone the gene that codes for it. Assume you know the sequence of this gene. Explain how you would go about this.

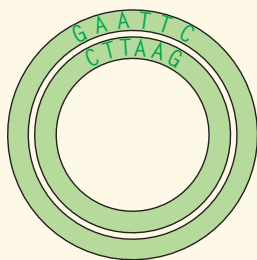
10. **MAKE CONNECTIONS** Looking at Figure 19.15, what does it mean for a SNP to be “linked” to a disease-associated allele? How does this allow the SNP to be used as a genetic marker? (See Concept 15.3.)



- 11. DRAW IT** You are cloning an aardvark gene, using a bacterial plasmid as a vector. The green diagram shows the plasmid, which contains the restriction site for the enzyme used in Figure 19.5. Above the plasmid is a segment of linear aardvark DNA that was synthesized using PCR. Diagram your cloning procedure, and show what would happen to these two molecules during each step. Use one color for the aardvark DNA and its bases and another color for those of the plasmid. Label each step and all 5' and 3' ends.

5' GAATTCTAAAGCGCTTATGAATTC 3'
3' CTTAAGATTTTCGCGAATACTTAAG 5'

Aardvark DNA



Plasmid

- 12. EVOLUTION CONNECTION** Ethical considerations aside, if DNA-based technologies became widely used, discuss how they might change the way evolution proceeds, as compared with the natural evolutionary mechanisms that have operated for the past 4 billion years.
- 13. SCIENTIFIC INQUIRY** In an experiment aimed at cloning pets, a group of scientists used nuclear transplantation. However, most of the progeny obtained from the experiment were excessively obese, carried many birth defects, and had

premature deaths. After a thorough check on the protocols followed, the scientists found some lacunae. What could have gone wrong with the experiment?

- 14. WRITE ABOUT A THEME: INFORMATION** In a short essay (100–150 words), discuss how the genetic basis of life plays a central role in biotechnology.

15. SYNTHESIZE YOUR KNOWLEDGE



The water in the Yellowstone National Park hot springs shown here is around 160°F (70°C). Biologists assumed that no species of organisms could live in water above about 130°F (55°C), so they were surprised to find several species of bacteria there, now called *thermophiles*

(“heat-lovers”). You’ve learned in this chapter how an enzyme from one species, *Thermus aquaticus*, made feasible one of the most important DNA-based techniques used in labs today. Identify the enzyme, and indicate the value of its being isolated from a thermophile. Suggest other reasons why enzymes from this bacterium (or other thermophiles) might also be valuable.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

The Evolution of Genomes

20



▲ **Figure 20.1** What genomic information distinguishes a human from a chimpanzee?

KEY CONCEPTS

- 20.1** The Human Genome Project fostered development of faster, less expensive sequencing techniques
- 20.2** Scientists use bioinformatics to analyze genomes and their functions
- 20.3** Genomes vary in size, number of genes, and gene density
- 20.4** Multicellular eukaryotes have a lot of noncoding DNA and many multigene families
- 20.5** Duplication, rearrangement, and mutation of DNA contribute to genome evolution
- 20.6** Comparing genome sequences provides clues to evolution and development



Reading the Leaves from the Tree of Life

The chimpanzee (*Pan troglodytes*) is our closest living relative on the evolutionary tree of life. The boy in **Figure 20.1** and his chimpanzee companion are intently studying the same leaf, but only one of them is able to talk about it. What accounts for this difference between two primates that share so much of their evolutionary history? With advances in sequencing technology, we are now addressing the genetic basis of such intriguing questions. Later in the chapter, you'll learn about the *FOXP2* gene involved in vocalization, which differs between the two species.

The chimpanzee genome was sequenced two years after sequencing of the human genome. Now that we can compare our genome, base by base, with that of the chimpanzee, we can tackle the more general issue of what differences in genetic information account for the distinct characteristics of these two species of primates.

In addition to determining the sequences of the human and chimpanzee genomes, researchers have obtained complete genome sequences for *Escherichia coli* (*E. coli*) and numerous other prokaryotes, as well as many eukaryotes, including *Zea mays* (corn), *Drosophila melanogaster* (fruit fly), *Octopus bimaculoides* (California two-spot octopus), and *Callorhynchus milii* (elephant shark; see the small photo). In 2014, a high-quality sequence was announced for the genome of *Homo neanderthalensis*, (Neanderthals), an extinct species closely related to present-day humans. These genomes are of great interest in their own right, but they also provide important insights into evolution as well as other biological processes. Broadening the

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 **Get Ready for This Chapter**

◀ **Elephant shark (*Callorhynchus milii*)**

human-chimpanzee comparison to the genomes of other primates and more distantly related animals should reveal the sets of genes that control group-defining characteristics. Beyond that, comparisons with the genomes of bacteria, archaea, fungi, protists, and plants should enlighten us about the long evolutionary history of the ancient genes we all share.

With the genomes of many species fully sequenced, scientists can study whole sets of genes and their interactions, an approach called **genomics**. The sequencing efforts that feed this approach have generated, and continue to generate, enormous volumes of data. The need to deal with this ever-increasing flood of information has spawned the field of **bioinformatics**, the application of computational methods to store and analyze biological data.

We will begin this chapter by discussing two approaches to genome sequencing and some of the advances in bioinformatics and its applications. We will then summarize what has been learned from the genomes that have been sequenced thus far. Next, we will describe the composition of the human genome as a representative genome of a complex multicellular eukaryote. Finally, we will explore current ideas about how genomes evolve and about how the evolution of developmental mechanisms could have generated the great diversity of life on Earth today.

CONCEPT 20.1

The Human Genome Project fostered development of faster, less expensive sequencing techniques

Sequencing of the human genome, an ambitious undertaking, officially began as the **Human Genome Project** in 1990. Organized by an international, publicly funded consortium of scientists at universities and research institutes, the project involved 20 large sequencing centers in six countries plus a host of other labs working on smaller parts of the project.

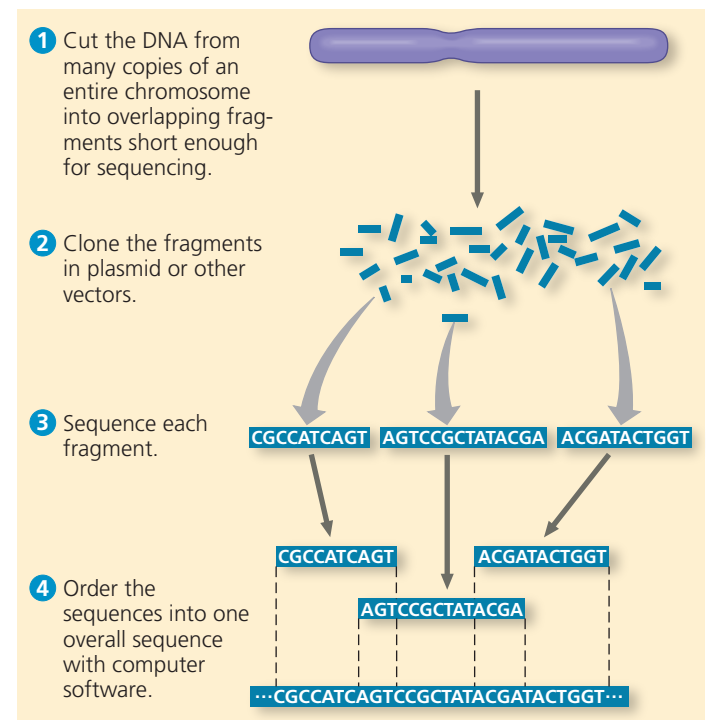
After the human genome sequence was largely completed in 2003, the sequence of each chromosome was analyzed and described in a series of papers, the last of which covered chromosome 1 and was published in 2006. At that point, the sequencing was considered “virtually complete.”

The ultimate goal in mapping any genome is to determine the complete nucleotide sequence of each chromosome. For the human genome, this was accomplished by sequencing machines using the dideoxy chain termination method mentioned in Concept 19.1. Even with this initial use of automation, though, the sequencing of all 3 billion base pairs in a haploid set of human chromosomes presented a formidable challenge. In fact, a major thrust of the Human Genome Project was the development of technology for faster sequencing (see Concept 19.1). Improvements over the years chipped away at each time-consuming step, enabling the rate of sequencing

to accelerate impressively: Whereas a productive lab could typically sequence 1,000 base pairs a day in the 1980s, by the year 2000 each research center working on the Human Genome Project was sequencing 1,000 base pairs *per second*. As of 2016, the most widely used automated machines can sequence nearly 25 million base pairs per second, while developers of some newer techniques claim they can achieve a rate of 66 billion base pairs per second. Methods that can analyze biological materials very rapidly and produce enormous volumes of data are said to be “high-throughput.” Sequencing machines are an example of high-throughput devices.

Two approaches complemented each other in obtaining the complete sequence. The initial approach was a methodical one that built on an earlier storehouse of human genetic information. In 1998, however, molecular biologist J. Craig Venter set up a company (Celera Genomics) and declared his intention to sequence the entire human genome using an alternative strategy. The **whole-genome shotgun approach** starts with the cloning and sequencing of DNA fragments from randomly cut DNA. Powerful computer programs then assemble the resulting very large number of overlapping short sequences into a single continuous sequence (**Figure 20.2**).

✓ Figure 20.2 Whole-genome shotgun approach to sequencing. In this approach, developed by J. Craig Venter and colleagues at Celera Genomics, random DNA fragments are cloned (see Figure 19.4), sequenced, and then ordered relative to each other.



VISUAL SKILLS ▶ The fragments in step 2 of this figure are depicted as scattered, rather than arranged in an ordered array. How does this depiction reflect the approach?



HMMI Animation: Shotgun Sequencing



Today, the whole-genome shotgun approach is still used, although newer, “next-generation” sequencing techniques (see Figure 19.3) have resulted in massive increases in speed and decreases in the cost of sequencing entire genomes. In these new techniques, many very small DNA fragments (each about 300 base pairs long) are sequenced at the same time, and computer software rapidly assembles the complete sequence. Because of the sensitivity of these techniques, the fragments can be sequenced directly; the cloning step (2 in Figure 20.2) is unnecessary. Whereas sequencing the first human genome took 13 years and cost \$100 million, the genome of James Watson (co-discoverer of DNA structure) was sequenced using newer techniques in four months in 2007 for about \$1 million, and as of 2016, an individual’s genome can be sequenced in a day or so for about \$1,000.

These technological advances have also facilitated an approach called **metagenomics** (from the Greek *meta*, beyond), in which DNA from an entire community of species (a *metagenome*) is collected from an environmental sample and sequenced. Again, computer software sorts out the partial sequences and assembles them into the individual specific genomes. An advantage of this technique is the ability to sequence the DNA of mixed microbial populations, which eliminates the need to culture each species separately in the lab, a difficulty that has limited the study of microbes. So far, this approach has been applied to communities found in environments as diverse as the human intestine and ancient soils in the Arctic, where a 2014 study characterized dozens of species living together as a community as long as 50,000 years ago, including animals and plants as well as microbes.

At first glance, genome sequences of humans and other organisms are simply dry lists of nucleotide bases—millions of A’s, T’s, C’s, and G’s in mind-numbing succession. Making sense of this massive amount of data has called for new analytical approaches, which we discuss next.

CONCEPT CHECK 20.1

1. Describe the whole-genome shotgun approach.

For suggested answers, see Appendix A.

CONCEPT 20.2

Scientists use bioinformatics to analyze genomes and their functions

Each of the 20 or so sequencing centers around the world working on the Human Genome Project churned out voluminous amounts of DNA sequence day after day. As the data began to accumulate, the need to coordinate efforts to keep track of all the sequences became clear. Thanks to the foresight of research scientists and government officials involved

in the Human Genome Project, its goals included establishing centralized databases and refining analytical software, all to be made readily accessible on the Internet.



HHMI Video: Leading Edge Bioinformatics



Centralized Resources for Analyzing Genome Sequences

Making bioinformatics resources available to researchers worldwide and speeding up the dissemination of information served to accelerate progress in DNA sequence analysis. For example, in 1988, in preparation for the Human Genome Project in the United States, the National Library of Medicine (NLM) and the National Institutes of Health (NIH) joined forces to create an organization called the National Center for Biotechnology Information (NCBI), which today maintains a website (www.ncbi.nlm.nih.gov) with extensive resources useful for bioinformatics. On this site are links to databases, software, and a wealth of information about genomics and related topics. Similar websites have also been established by three genome centers with which the NCBI collaborates: the European Molecular Biology Laboratory, the DNA Data Bank of Japan, and BGI (formerly known as the Beijing Genomics Institute) in Shenzhen, China. These large, comprehensive websites are complemented by others maintained by individual or small groups of laboratories. Smaller websites often provide databases and software designed for a narrower purpose, such as studying genetic and genomic changes in one particular type of cancer.

The NCBI database of sequences is called GenBank. As of June 2016, it included the sequences of 194 million fragments of genomic DNA, totaling 213 billion base pairs! GenBank is constantly updated, and the amount of data it contains increases rapidly. Any sequence in the database can be retrieved and analyzed using software from the NCBI website or elsewhere.

One very widely used software program available on the NCBI website, called BLAST, allows the user to compare a DNA sequence with every sequence in GenBank, base by base. A researcher might search for similar regions in other genes of the same species or among the genes of other species. Another program allows comparison of protein sequences. Yet a third can search any protein sequence for *conserved* (common) stretches of amino acids (domains) for which a function is known or suspected, and it can show a three-dimensional model of the domain alongside other relevant information (Figure 20.3). There is even a software program that can align and compare a collection of sequences, either nucleic acids or polypeptides, and diagram them in the form of an evolutionary tree based on the sequence relationships. (One such diagram is shown in Figure 20.17.)

Two research institutions, Rutgers University and the University of California, San Diego, also maintain a worldwide database of all three-dimensional protein structures that

▼ **Figure 20.3 Bioinformatics tools that are available on the Internet.**

A website maintained by the National Center for Biotechnology Information (NCBI) allows scientists and the public to access DNA and protein sequences and other stored data.

The site includes a link to a protein structure database (Conserved Domain Database, CDD) that can find and describe similar domains in related proteins, as well as software (Cn3D, “See in 3-D”) that displays models of domains. Some results are shown from a search for

regions of proteins similar to an amino acid sequence in a muskmelon protein. The WD40 domain is very common in proteins encoded by eukaryotic genomes. It often plays a key role in molecular interactions during signal transduction.

❶ In this window, a partial amino acid sequence from an unknown muskmelon protein (“Query”) is aligned with sequences from other proteins that the computer program found to be similar. Each sequence represents a domain called WD40.

❷ Four hallmarks of the WD40 domain are highlighted in yellow. (Sequence similarity is based on chemical aspects of the amino acids, so the amino acids in each hallmark region are not always identical.)

The image shows a screenshot of a web browser displaying two windows. The top window is titled "WD40 - Sequence Alignment Viewer" and shows a sequence alignment of a "Query" sequence (muskmelon protein) with several other sequences from different organisms: Cow [transducin], Mustard weed [transducin], Corn [GNB protein], Human [PAFA protein], Nematode [unknown protein #1], Nematode [unknown protein #2], and Fission yeast [FWDR protein]. The sequences are aligned, and four specific regions (hallmarks) are highlighted in yellow. The bottom window is titled "WD40 - Cn3D 4.1" and displays a 3D ribbon model of the cow transducin protein. The protein is shown in purple, and the four hallmarks are highlighted in yellow. A third window, titled "CDD Descriptive Items", is open on the left, showing information about the WD40 domain, including its function and structure.

❸ The Cn3D program displays a three-dimensional ribbon model of cow transducin (the protein highlighted in purple in the Sequence Alignment Viewer). This protein is the only one of those shown for which a structure has been determined. The sequence similarity of the other proteins to cow transducin suggests that their structures are likely to be similar.

❹ Cow transducin contains seven WD40 domains, one of which is highlighted here in gray.

❺ The yellow segments correspond to the WD40 hallmarks highlighted in yellow in the window above.

❻ This window displays information about the WD40 domain from the Conserved Domain Database.

have been experimentally determined, called the Protein Data Bank (www.wwpdb.org). These structures can be rotated by the viewer to show all sides of the protein. Throughout this book, you'll find images of protein structures that have been obtained from the Protein Data Bank.

There is a vast array of resources available for researchers anywhere in the world to use free of charge. Let us now consider the types of questions scientists can address using these resources.



Instructors: BLAST Data Analysis Tutorials, which teach students how to work with real data from the BLAST database, can be assigned in MasteringBiology.

Identifying Protein-Coding Genes and Understanding Their Functions

Using available DNA sequences, geneticists can study genes directly, rather than taking the classical genetic approach, which requires determining the function of an unknown gene from the phenotype. But this more recent approach poses a new challenge: What does the gene actually do? Given a long DNA sequence from a database like GenBank,

scientists aim to identify all protein-coding genes in the sequence and ultimately their functions. This process, called **gene annotation**, uses three lines of evidence to identify a gene.

First, computers are utilized in a search for patterns that indicate the presence of genes. The usual approach is to use software to scan the stored sequences for those that represent transcriptional and translational start and stop signals, RNA-splicing sites, and other telltale signs of protein-coding genes. The software also looks for certain short sequences that specify known mRNAs. Thousands of such sequences, called *expressed sequence tags*, or *ESTs*, have been collected from cDNA sequences and are cataloged in computer databases. This type of analysis identifies sequences that may turn out to be previously unknown protein-coding genes.

Although the identities of about half of the human genes were known before the Human Genome Project began, the other genes, previously unknown, were revealed by DNA sequence analysis. Once such suspected genes are identified, the second step is to obtain clues about their identities and functions by using software to compare their sequences with those of known genes from other organisms. Due to

redundancy in the genetic code, the DNA sequence itself may vary more among species than the protein sequence does. Thus, scientists interested in proteins often compare the predicted amino acid sequence of a protein to that of other proteins. Third, the identities of these genes must then be confirmed by using RNA-seq (see Figure 19.13) or some other method to show that the relevant RNA is actually expressed from the proposed gene.



Animation: The Human Genome Project: Genes on Human Chromosome 17

Sometimes a newly identified sequence will match, at least partially, the sequence of a gene or protein in another species whose function is well known. For example, a plant researcher working on signaling pathways in the muskmelon would be excited to see that a partial amino acid sequence from a gene she had identified matched sequences in other species encoding a functional part of a protein called a WD40 domain (see Figure 20.3). WD40 domains are present in many eukaryotic proteins and are known to function in signal transduction pathways. Alternatively, a new gene sequence might be similar to a previously encountered sequence whose function is still unknown. Another possibility is that the sequence is entirely unlike anything ever seen before. This was true for about a third of the genes of *E. coli* when its genome was sequenced. In such cases, protein function is usually deduced through a combination of biochemical and functional studies. The biochemical approach aims to determine the three-dimensional structure of the protein as well as other attributes, such as potential binding sites for other molecules. Functional studies usually involve *knocking out* (blocking or disabling) the gene in an organism to see how the phenotype is affected. The CRISPR-Cas 9 system, described in Figure 19.14, is an example of an experimental technique used to block gene function.

Understanding Genes and Gene Expression at the Systems Level

The impressive computational power provided by the tools of bioinformatics allows the study of whole sets of genes and their interactions, as well as the comparison of genomes from different species. Genomics is a rich source of new insights into fundamental questions about genome organization, regulation of gene expression, embryonic development, and evolution.

One informative approach was taken by a long-term research project called ENCODE (Encyclopedia of DNA Elements), which ran from 2003 to 2012. The aim of the project was to learn everything possible about the functionally important elements in the human genome using multiple experimental techniques on different types of cultured cells. Investigators sought to identify protein-coding genes and genes for non-coding RNAs, along with sequences that regulate gene expression, such as enhancers and promoters. In addition, they

extensively characterized DNA and histone modifications and chromatin structure—features termed “epigenetic” since they affect gene expression without changing the sequence of nucleotide bases (see Concept 18.3). The second phase of the project, involving more than 440 scientists in 32 research groups, culminated in 2012 with the simultaneous publication of 30 papers describing over 1,600 large data sets. The considerable power of this project is that it provides the opportunity to compare results from specific projects with each other, yielding a much richer picture of the whole genome.

Perhaps the most striking finding is that about 75% of the genome is transcribed at some point in at least one of the cell types studied, even though less than 2% codes for proteins. Furthermore, biochemical functions have been assigned to DNA elements making up at least 80% of the genome. To learn more about the different types of functional elements, parallel projects are analyzing in a similar way the genomes of two model organisms, the soil nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. Because genetic and biochemical experiments using DNA technology can be performed on these species, testing the activities of potentially functional DNA elements in their genomes is expected to illuminate the workings of the human genome.

Because the ENCODE project analyzed cells in culture, its potential for clinical applications was limited. A related project called the Roadmap Epigenomics Project set out to characterize the *epigenome*—the epigenetic features of the genome—of hundreds of human cell types and tissues. The aim was to focus on the epigenomes of stem cells, normal tissues from mature adults, and relevant tissues from individuals with diseases such as cancer and neurodegenerative and autoimmune disorders. In 2015, a series of papers reported on the results from 111 tissues. One of the most useful findings was that the original tissue in which a cancer arose can be identified in cells of a secondary tumor based on characterization of their epigenomes.

Systems Biology

The scientific progress resulting from sequencing genomes and studying large sets of genes has encouraged scientists to attempt similar systematic studies of sets of proteins and their properties (such as their abundance, chemical modifications, and interactions), an approach called **proteomics**. (A **proteome** is the entire set of proteins expressed by a cell or group of cells.) Proteins, not the genes that encode them, carry out most of the activities of the cell. Therefore, we must study when and where proteins are produced in an organism, as well as how they interact in networks, if we are to understand the functioning of cells and organisms.

Genomics and proteomics enable molecular biologists to approach the study of life from an increasingly global perspective. Using the tools we have described, biologists have begun to compile catalogs of genes and proteins—listings of all the “parts” that contribute to the operation of cells,

tissues, and organisms. With such catalogs in hand, researchers have shifted their attention from the individual parts—the genes and proteins—to their functional integration in biological systems. As you may recall, Concept 1.1 discussed this approach, called **systems biology**, which aims to model the dynamic behavior of whole biological systems based on the study of the interactions among the system's parts. Because of the vast amounts of data generated in these types of studies, advances in computer technology and bioinformatics are crucial to studying systems biology.

One important use of the systems biology approach is to define gene and protein interaction networks. To map the protein interaction network in the yeast *Saccharomyces cerevisiae*, for instance, researchers used sophisticated techniques to knock out pairs of genes, one pair at a time, creating doubly mutant cells. They then compared the fitness of each double mutant (based in part on the size of the cell colony it formed) to that predicted from the fitness of each of the two single mutants. The researchers reasoned that if the observed fitness matched the prediction, then the products of the two genes didn't interact with each other, but if the observed fitness was greater or less than predicted, then the gene products interacted in the cell. They then used computer software to build a graphic model by “mapping” the gene products to certain locations in the model, based on the similarity of their interactions. This resulted in the network-like “functional map” of protein interactions shown in **Figure 20.4**. Processing the vast number of protein-protein interactions generated by this experiment

and integrating them into the completed map required powerful computers, mathematical tools, and newly developed software.

Application of Systems Biology to Medicine

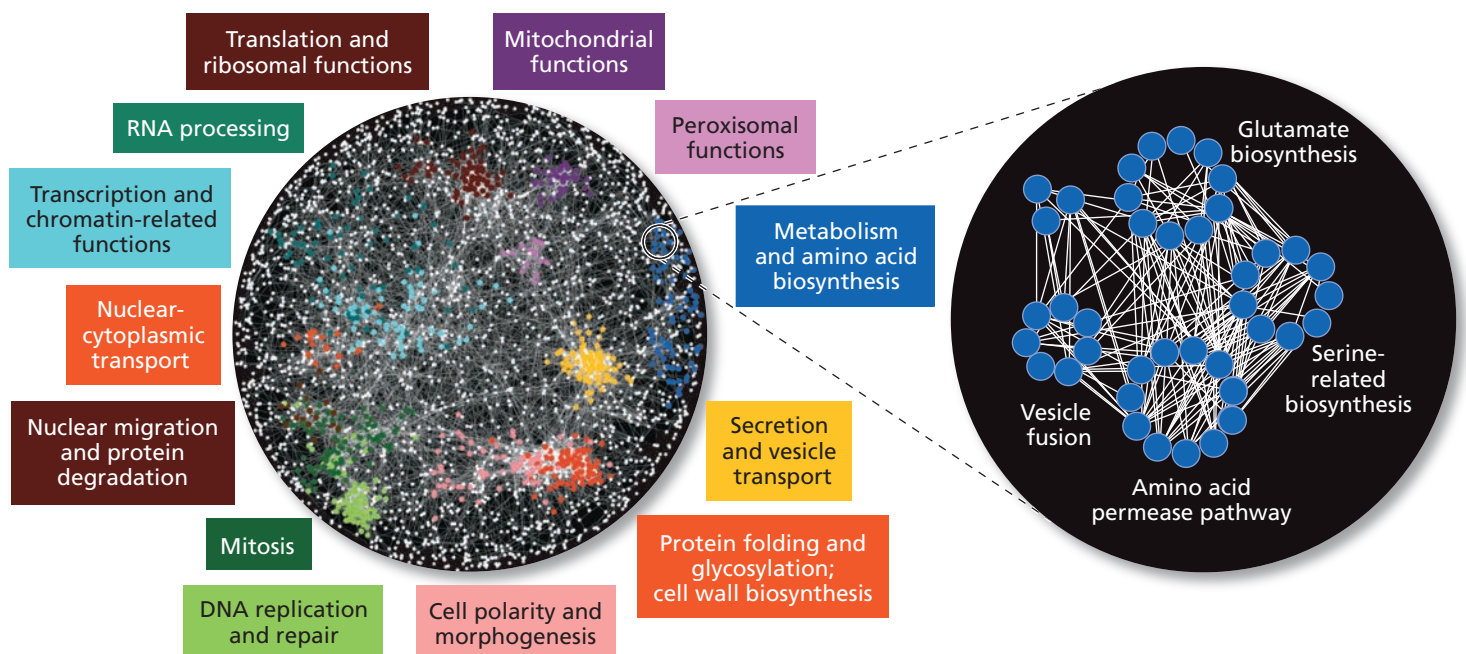
The Cancer Genome Atlas is another example of systems biology in which many interacting genes and gene products are analyzed together, as a group. This project, under the joint leadership of the National Cancer Institute and the NIH, aims to determine how changes in biological systems lead to cancer. A three-year pilot project that ended in 2010 set out to find all the common mutations in three types of cancer—lung cancer, ovarian cancer, and glioblastoma of the brain—by comparing gene sequences and patterns of gene expression in cancer cells with those in normal cells. Work on glioblastoma confirmed the role of several suspected genes and identified a few previously unknown ones, suggesting possible new targets for therapies. The approach proved so fruitful for these three types of cancer that it has been extended to ten other types, chosen because they are common and often lethal in humans.

As high-throughput techniques become more rapid and less expensive, they are being increasingly applied to the problem of cancer, as seen with the Roadmap Epigenomics Project described earlier. Rather than sequencing only protein-coding genes, sequencing the whole genomes of many tumors of a particular type allows scientists to uncover common chromosomal abnormalities, as well as any other consistent changes in these aberrant genomes.

▼ **Figure 20.4 The systems biology approach to protein interactions.** This global protein interaction map shows the likely interactions (lines) among about 4,500 gene products (dots) in *Saccharomyces cerevisiae*,

the budding yeast. Dots of the same color represent gene products involved in one of the 13 similarly colored cellular functions listed around the map. The white dots represent proteins that haven't been assigned to any

color-coded function. The expanded area shows additional details of one map region where the gene products (blue dots) carry out amino acid biosynthesis, uptake, and related functions.





◀ **Figure 20.5 A human gene microarray chip.** Tiny spots of DNA arranged in a grid on this silicon wafer represent almost all of the genes in the human genome. Using this chip, researchers can analyze expression patterns for all these genes at the same time (see Figure 19.12).



HHMI Animation: Gene Chip Manufacturing



In addition to whole-genome sequencing, silicon and glass “chips” that hold a microarray of most of the known human genes are now used to analyze gene expression patterns in patients suffering from various cancers and other diseases (**Figure 20.5**). Increasingly, RNA-seq (see Figure 19.13) is replacing microarray analysis. Analyzing which genes are over- or underexpressed in a particular cancer may allow physicians to tailor patients’ treatment to their unique genetic makeup and the specifics of their cancers. This approach has been used to characterize subsets of particular cancers, enabling more refined treatments. Breast cancer is one example (see Figure 18.27).

Ultimately, medical records may include an individual’s DNA sequence, a sort of genetic bar code, with regions highlighted that predispose the person to specific diseases. The use of such sequences for personalized medicine—disease prevention and treatment—has great potential.

Systems biology is a very efficient way to study emergent properties at the molecular level. Novel properties arise at each successive level of biological complexity as a result of the arrangement of building blocks at the underlying level (see Concept 1.1). The more we can learn about the arrangement and interactions of the components of genetic systems, the deeper will be our understanding of whole organisms. The rest of this chapter surveys what we’ve learned from genomic studies.

CONCEPT CHECK 20.2

1. What are the ways by which the functions of newly sequenced genes can be ascertained?
2. Explain the advantage of the systems biology approach to studying cancer versus the approach of studying a single gene at a time.
3. **MAKE CONNECTIONS** ▶ The ENCODE pilot project found that at least 75% of the genome is transcribed into RNAs, far more than could be accounted for by protein-coding genes. Review Concepts 17.3 and 18.3 and suggest some roles that these RNAs might play.
4. **MAKE CONNECTIONS** ▶ In Concept 19.2, you learned about genome-wide association studies. Explain how these studies use the systems biology approach.

For suggested answers, see Appendix A.

CONCEPT 20.3

Genomes vary in size, number of genes, and gene density

The sequences of thousands of genomes have been completed, with tens of thousands of genomes either in progress or considered permanent drafts (because they require more work than it would be worth to complete them). Among the sequences in progress are roughly 3,400 metagenomes. In the completely sequenced group, about 5,000 are genomes of bacteria, and 242 are archaeal genomes. There are 283 completed eukaryotic species, along with 2,635 permanent drafts. Among these are vertebrates, invertebrates, protists, fungi, and plants. Next, we’ll discuss what we’ve learned about genome size, number of genes, and gene density, focusing on general trends.

Genome Size

Comparing the three domains (Bacteria, Archaea, and Eukarya), we find a general difference in genome size between prokaryotes and eukaryotes (**Table 20.1**). While there are

Table 20.1 Genome Sizes and Estimated Numbers of Genes*

Organism	Haploid Genome Size (Mb)	Number of Genes	Genes per Mb
Bacteria			
<i>Haemophilus influenzae</i>	1.8	1,700	940
<i>Escherichia coli</i>	4.6	4,400	950
Archaea			
<i>Archaeoglobus fulgidus</i>	2.2	2,500	1,130
<i>Methanosarcina barkeri</i>	4.8	3,600	750
Eukaryotes			
<i>Saccharomyces cerevisiae</i> (yeast, a fungus)	12	6,300	525
<i>Urticularia gibba</i> (floating bladderwort)	82	28,500	348
<i>Caenorhabditis elegans</i> (nematode)	100	20,100	200
<i>Arabidopsis thaliana</i> (mustard family plant)	120	27,000	225
<i>Drosophila melanogaster</i> (fruit fly)	165	14,000	85
<i>Daphnia pulex</i> (water flea)	200	31,000	155
<i>Zea mays</i> (corn)	2,300	32,000	14
<i>Ailuropoda melanoleuca</i> (giant panda)	2,400	21,000	9
<i>Homo sapiens</i> (human)	3,000	< 21,000	7
<i>Paris japonica</i> (Japanese canopy plant)	149,000	ND	ND

*Some values given here are likely to be revised as genome analysis continues. Mb = million base pairs. ND = not determined.

some exceptions, most bacterial genomes have between 1 and 6 million base pairs (Mb); the genome of *E. coli*, for instance, has 4.6 Mb. Genomes of archaea are, for the most part, within the size range of bacterial genomes. (Keep in mind, however, that many fewer archaeal genomes have been completely sequenced, so this picture may change.) Eukaryotic genomes tend to be larger: The genome of the single-celled yeast *Saccharomyces cerevisiae* (a fungus) has about 12 Mb, while most animals and plants, which are multicellular, have genomes of at least 100 Mb. There are 165 Mb in the fruit fly genome, while humans have 3,000 Mb, about 500 to 3,000 times as many as a typical bacterium.

Aside from this general difference between prokaryotes and eukaryotes, a comparison of genome sizes among eukaryotes fails to reveal any systematic relationship between genome size and the organism's phenotype. For instance, the genome of *Paris japonica*, the Japanese canopy plant, contains 149 billion base pairs (149,000 Mb), while that of another plant, *Urticularia gibba*, a bladderwort, contains only 82 Mb. Even more striking, there is a single-celled amoeba, *Polychaos dubium*, whose genome size has been estimated at 670 billion base pairs (670,000 Mb). (This genome has not yet been sequenced.) On a finer scale, comparing two insect species, the cricket (*Anabrus simplex*) genome turns out to have 11 times as many base pairs as the fruit fly (*Drosophila melanogaster*) genome. There is a wide range of genome sizes within the groups of unicellular eukaryotes, insects, amphibians, and plants, and less of a range within mammals and reptiles.

Number of Genes

The number of genes also varies between prokaryotes and eukaryotes: Bacteria and archaea, in general, have fewer genes than eukaryotes. Free-living bacteria and archaea have from 1,500 to 7,500 genes, while the number of genes in eukaryotes ranges from about 5,000 for unicellular fungi (yeasts) to at least 40,000 for some multicellular eukaryotes.

Within the eukaryotes, the number of genes in a species is often lower than expected from considering simply the size of its genome. Looking at Table 20.1, you can see that the genome of the nematode *C. elegans* is 100 Mb in size and contains roughly 20,100 genes. In comparison, the genome of *Drosophila melanogaster* is much bigger (165 Mb) but has only about two-thirds the number of genes—14,000 genes.

Considering an example closer to home, we noted that the human genome contains 3,000 Mb, well over ten times the size of either the *D. melanogaster* or *C. elegans* genome. At the outset of the Human Genome Project, biologists expected somewhere between 50,000 and 100,000 genes to be identified in the completed sequence, based on the number of known human proteins. As the project progressed, the estimate was revised downward several times, and the ENCODE

project discussed above has established the number to be fewer than 21,000. This relatively low number, similar to the number of genes in the nematode *C. elegans*, surprised biologists, who had been expecting many more human genes.



Interview with Eric Lander: Exploring the Human Genome

What genetic attributes allow humans (and other vertebrates) to get by with no more genes than nematodes? An important factor is that vertebrate genomes “get more bang for the buck” from their coding sequences because of extensive alternative splicing of RNA transcripts. Recall that this process generates more than one polypeptide from a single gene (see Figure 18.13). A typical human gene contains about ten exons, and an estimated 90% or more of these multi-exon genes are spliced in at least two different ways. Some genes are expressed in hundreds of alternatively spliced forms, others in just two. Scientists have not yet catalogued all of the different forms, but it is clear that the number of different proteins encoded in the human genome far exceeds the proposed number of genes.

Additional polypeptide diversity could result from post-translational modifications such as cleavage or the addition of carbohydrate groups in different cell types or at different developmental stages. Finally, the discovery of miRNAs and other small RNAs that play regulatory roles has added a new variable to the mix (see Concept 18.3). Some scientists think that this added level of regulation, when present, may contribute to greater organismal complexity for some genes.

Gene Density and Noncoding DNA

We can take both genome size and number of genes into account by comparing gene density in different species. In other words, we can ask: How many genes are in a given length of DNA? When we compare the genomes of bacteria, archaea, and eukaryotes, we see that eukaryotes generally have larger genomes but fewer genes in a given number of base pairs. Humans have hundreds or thousands of times as many base pairs in their genome as most bacteria, as we already noted, but only 5 to 15 times as many genes; thus, gene density is lower in humans (see Table 20.1). Even unicellular eukaryotes, such as yeasts, have fewer genes per million base pairs than bacteria and archaea. Among the genomes that have been sequenced completely, humans and other mammals have the lowest gene density.

In all bacterial genomes studied so far, most of the DNA consists of genes for protein, tRNA, or rRNA; the small amount remaining consists mainly of nontranscribed regulatory sequences, such as promoters. The sequence of nucleotides along a bacterial protein-coding gene is not interrupted by noncoding sequences (introns). In eukaryotic genomes, by contrast, most of the DNA neither encodes protein nor is transcribed into RNA molecules of known function, and

the DNA includes more complex regulatory sequences. In fact, humans have 10,000 times as much noncoding DNA as bacteria. Some of this DNA in multicellular eukaryotes is present as introns within genes. Indeed, introns account for most of the difference in average length between human genes (27,000 base pairs) and bacterial genes (1,000 base pairs).

In addition to introns, multicellular eukaryotes have a vast amount of non-protein-coding DNA between genes. In the next section, we will describe the composition and arrangement of these great stretches of DNA in the human genome.

CONCEPT CHECK 20.3

1. Eukaryotes such as humans have around 21,000 genes and 7 genes per megabase. Prokaryotes such as *E. coli*, on the other hand, have 4,400 genes and 950 genes per megabase. What accounts for the lower gene density in eukaryotes?
2. The Genomes Online Database (GOLD) website of the Joint Genome Institute has information about genome sequencing projects. Scroll through the page at <https://gold.jgi.doe.gov/statistics> and describe the information you find. What percent of bacterial genome projects have medical relevance?
3. **WHAT IF?** > What evolutionary processes might account for prokaryotes having smaller genomes than eukaryotes?

For suggested answers, see Appendix A.

CONCEPT 20.4

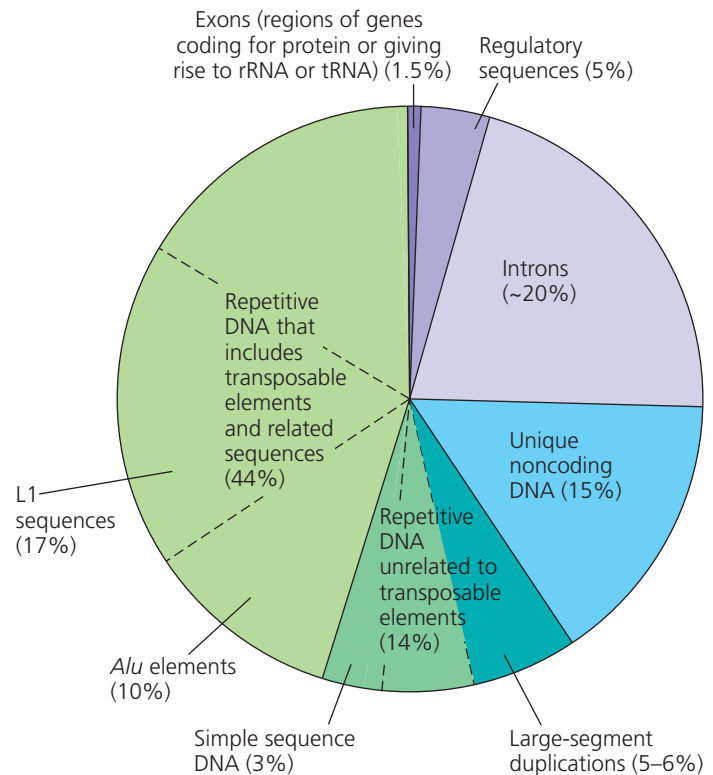
Multicellular eukaryotes have a lot of noncoding DNA and many multigene families

We have spent most of this chapter, and indeed this unit, focusing on genes that code for proteins. Yet the coding regions of these genes and the genes for noncoding RNA products such as rRNA, tRNA, and miRNA make up only a small portion of the genomes of most multicellular eukaryotes. For example, once the sequencing of the human genome was completed, it became clear that only a tiny part—about 1.5%—codes for proteins or is transcribed into rRNAs or tRNAs. **Figure 20.6** shows what is known about the makeup of the remaining 98.5% of the genome.

Gene-related regulatory sequences and introns account, respectively, for 5% and about 20% of the human genome. The rest, located between functional genes, includes some unique (single-copy) noncoding DNA, such as gene fragments and **pseudogenes**, former genes that have accumulated mutations over a long time and no longer produce functional proteins. (The genes that produce small noncoding RNAs are a tiny percentage of the genome, distributed between the 20% introns and the 15% unique noncoding DNA.) Most of the DNA between functional genes, however,

▼ Figure 20.6 Types of DNA sequences in the human genome.

The gene sequences that code for proteins or are transcribed into rRNA or tRNA molecules make up only about 1.5% of the human genome (dark purple in the pie chart), while introns and regulatory sequences associated with genes (light purple) make up about a quarter. The vast majority of the human genome does not code for proteins (although much of it gives rise to RNAs), and a large amount is repetitive DNA (dark and light green and teal).



is **repetitive DNA**, which consists of sequences that are present in multiple copies in the genome.

The bulk of many eukaryotic genomes consists of DNA sequences that neither code for proteins nor are transcribed to produce RNAs with known functions; this noncoding DNA was often described in the past as “junk DNA.” However, genome comparisons over the past 10 years have revealed the persistence of this DNA in diverse genomes over many hundreds of generations. For example, the genomes of humans, rats, and mice contain almost 500 regions of noncoding DNA that are *identical* in sequence in all three species. This is a higher level of sequence conservation than is seen for protein-coding regions in these species, strongly suggesting that the noncoding regions have important functions. The results of the ENCODE project discussed earlier have underscored the key roles played by much of this noncoding DNA. In the next few pages, we examine how genes and noncoding DNA sequences are organized within genomes of multicellular eukaryotes, using the human genome as our main example. Genome organization tells us a lot about how genomes have evolved and continue to evolve, as we’ll discuss in Concept 20.5.

Transposable Elements and Related Sequences

Both prokaryotes and eukaryotes have stretches of DNA that can move from one location to another within the genome. These stretches are known as *transposable genetic elements*, or simply **transposable elements**. During the process called *transposition*, a transposable element moves from one site in a cell's DNA to a different target site by a type of recombination process. Transposable elements are sometimes called “jumping genes,” but actually they never completely detach from the cell's DNA. Instead, the original and new DNA sites are brought very close together by enzymes and other proteins that bend the DNA. Surprisingly, about 75% of human repetitive DNA (44% of the entire human genome) is made up of transposable elements and sequences related to them.

The first evidence for wandering DNA segments came from American geneticist Barbara McClintock's breeding experiments with Indian corn (maize) in the 1940s and 1950s (**Figure 20.7**). As she tracked corn plants through multiple generations, McClintock identified changes in the color of corn kernels that made sense only if she postulated the existence of genetic elements capable of moving from other locations in the genome into the genes for kernel color, disrupting the genes so that the kernel color was changed. McClintock's discovery was met with great skepticism and virtually discounted at the time. Her careful work and insightful ideas were finally validated many years later when transposable elements were found in bacteria. In 1983, at the age of 81, McClintock received the Nobel Prize for her pioneering research.

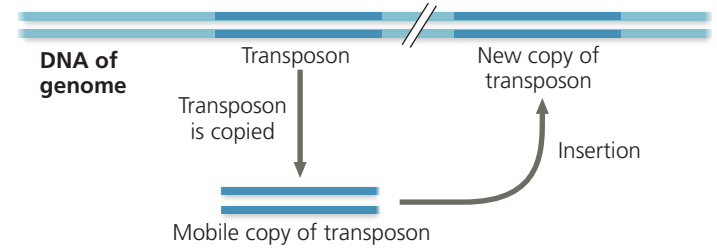
Movement of Transposons and Retrotransposons

Eukaryotic transposable elements are of two types. The first type, **transposons**, move within a genome by means of a



◀ **Figure 20.7** The effect of transposable elements on corn kernel color. Barbara McClintock first proposed the idea of mobile genetic elements after observing variegations in the color of the kernels on a corn cob (top right).

▼ **Figure 20.8** Transposon movement. Movement of transposons by either the copy-and-paste mechanism (shown here) or the cut-and-paste mechanism involves a double-stranded DNA intermediate that is inserted into the genome.



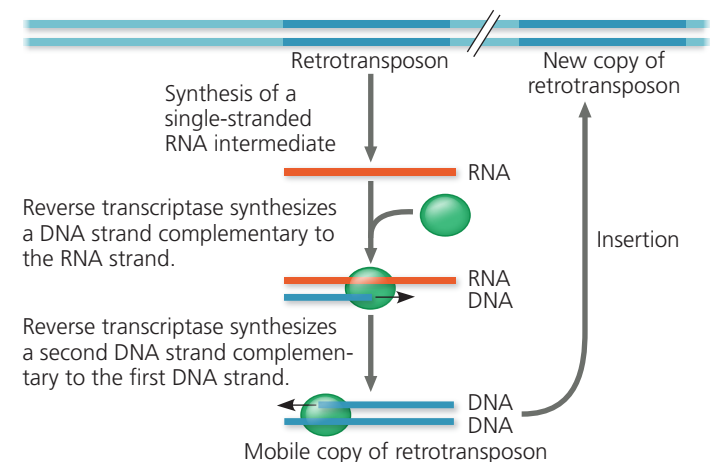
VISUAL SKILLS ► How would this figure differ if it showed the cut-and-paste mechanism?

MB Interview with Virginia Walbot: Plant genetics and development

DNA intermediate. Transposons can move by a “cut-and-paste” mechanism, which removes the element from the original site, or by a “copy-and-paste” mechanism, which leaves a copy behind (**Figure 20.8**). Both mechanisms require an enzyme called *transposase*, which is generally encoded by the transposon.

Most transposable elements in eukaryotic genomes are of the second type, **retrotransposons**, which move by means of an RNA intermediate that is a transcript of the retrotransposon DNA. Thus, retrotransposons always leave a copy at the original site during transposition (**Figure 20.9**). To insert at another site, the RNA intermediate is first converted back to DNA by reverse transcriptase, an enzyme encoded by the retrotransposon. (Reverse transcriptase is also encoded by retroviruses, which are discussed in Concept 26.2. In fact, retroviruses may have evolved from retrotransposons.) Another cellular enzyme catalyzes insertion of the reverse-transcribed DNA at a new site.

▼ **Figure 20.9** Retrotransposon movement. Movement begins with synthesis of a single-stranded RNA intermediate. The remaining steps are essentially identical to part of the retrovirus replicative cycle (see Figure 26.8).



Sequences Related to Transposable Elements

Multiple copies of transposable elements and sequences related to them are scattered throughout eukaryotic genomes. A single unit is usually hundreds to thousands of base pairs long, and the dispersed copies are similar but usually not identical to each other. Some of these are transposable elements that can move; the enzymes required for this movement may be encoded by any transposable element, including the one that is moving. Others are related sequences that have lost the ability to move altogether. Transposable elements and related sequences make up 25–50% of most mammalian genomes (see Figure 20.6) and even higher percentages in amphibians and many plants. In fact, the very large size of some plant genomes is accounted for by extra transposable elements rather than by extra genes. For example, transposable elements make up 85% of the corn genome!

In humans and other primates, a large portion of transposable element-related DNA consists of a family of similar sequences called *Alu elements*. These sequences alone account for approximately 10% of the human genome. *Alu* elements are about 300 nucleotides long, much shorter than most functional transposable elements, and they do not code for any protein. However, many *Alu* elements are transcribed into RNA, and at least some of these RNAs are thought to help regulate gene expression.

An even larger percentage (17%) of the human genome is made up of a type of retrotransposon called *LINE-1*, or *L1*. These sequences are much longer than *Alu* elements—about 6,500 base pairs—and typically have a very low rate of transposition. However, researchers working with rats have found *L1* retrotransposons to be more active in cells of the developing brain. They have proposed that different effects on gene expression of *L1* retrotransposition in developing neurons may contribute to the great diversity of neuronal cell types (see Concept 48.1).

Although many transposable elements encode proteins, these proteins do not carry out normal cellular functions. Therefore, transposable elements are usually included in the “noncoding” DNA category, along with other repetitive sequences.

Other Repetitive DNA, Including Simple Sequence DNA

Repetitive DNA that is not related to transposable elements has probably arisen from mistakes during DNA replication or recombination. Such DNA accounts for about 14% of the human genome (see Figure 20.6). About a third of this (5–6% of the human genome) consists of duplications of long stretches of DNA, with each unit ranging from 10,000 to 300,000 base pairs. These long segments seem to have been copied from one chromosomal location to another site on the same or a different chromosome and probably include some functional genes.

In contrast to scattered copies of long sequences, stretches of DNA known as **simple sequence DNA** contain many copies of tandemly repeated short sequences, as in the following example (showing one DNA strand only):

... GTTACGTTACGTTACGTTACGTTACGTTAC ...

In this case, the repeated unit (GTTAC) consists of 5 nucleotides. Repeated units may contain as many as 500 nucleotides, but often contain fewer than 15 nucleotides, as in this example. When the unit contains 2–5 nucleotides, the series of repeats is called a **short tandem repeat**, or **STR**; we discussed the use of STR analysis in preparing genetic profiles in Concept 19.4 (see Figure 19.24). The number of copies of the repeated unit can vary from site to site within a given genome. There could be as many as several hundred thousand repetitions of the GTTAC unit at one site, but only half that number at another. STR analysis is performed on sites selected because they have relatively few repeats. The repeat number varies from person to person, and since humans are diploid, each person has two alleles per site, which can differ in repeat number. This diversity produces the variation represented in the genetic profiles that result from STR analysis. Altogether, simple sequence DNA makes up 3% of the human genome.

Much of a genome’s simple sequence DNA is located at chromosomal telomeres and centromeres, suggesting that this DNA plays a structural role for chromosomes. The DNA at centromeres is essential for the separation of chromatids in cell division (see Concept 12.2). Centromeric DNA, along with simple sequence DNA located elsewhere, may also help organize the chromatin within the interphase nucleus. The simple sequence DNA located at telomeres—the tips of chromosomes—prevents genes from being lost as the DNA shortens with each round of replication (see Concept 16.2). Telomeric DNA also binds proteins that protect the ends of a chromosome from degradation and from joining to other chromosomes.

Short repetitive sequences like those described here provide a challenge for whole-genome shotgun sequencing because the presence of many short repeats hinders accurate reassembly of fragment sequences by computers. Regions of simple sequence DNA account for much of the uncertainty present in estimates of whole-genome sizes and are the reason some sequences are considered “permanent drafts.”

Genes and Multigene Families

We finish our discussion of the various types of DNA sequences in eukaryotic genomes with a closer look at genes. Recall that DNA sequences that code for proteins or give rise to tRNA or rRNA compose a mere 1.5% of the human genome (see Figure 20.6). If we include introns and regulatory sequences associated with genes, the total amount of DNA that is gene-related—coding and noncoding—constitutes about 25% of the human genome. Put another way, only about 6% (1.5% out of 25%) of the length of the average gene is represented in the final gene product.

Like the genes of bacteria, many eukaryotic genes are present as unique sequences, with only one copy per haploid set of chromosomes. But in the human genome and the genomes of many other animals and plants, these unique genes make up less than half of the total gene-related DNA. The rest occur in **multigene families**, collections of two or more identical or very similar genes.

In multigene families that consist of *identical* DNA sequences, those sequences are usually clustered tandemly and, with the notable exception of the genes for histone proteins, have RNAs as their final products. An example is the family of identical DNA sequences that each include the genes for the three largest rRNA molecules (**Figure 20.10a**). These rRNA molecules are transcribed from a single transcription unit that is repeated tandemly hundreds to thousands of times in one or several clusters in the genome of a multicellular eukaryote. The many copies of this rRNA transcription unit help cells to quickly make the millions of ribosomes needed for active protein synthesis. The primary transcript is cleaved to yield three rRNA molecules, which combine with proteins and one other kind of rRNA (5S rRNA) to form ribosomal subunits.

The classic examples of multigene families of *nonidentical* genes are two related families of genes that encode globins, a group of proteins that include the α and β polypeptide subunits of hemoglobin. One family, located on chromosome 16 in humans, encodes various forms of α -globin; the other, on chromosome 11, encodes forms of β -globin (**Figure 20.10b**). The different forms of each globin subunit are expressed at different times in development, allowing hemoglobin to function effectively in the changing environment of the developing animal. In humans, for example, the embryonic and fetal forms of hemoglobin have a higher affinity for oxygen than the adult forms, ensuring the efficient transfer of oxygen from mother to fetus. Also found in the globin gene family clusters are several pseudogenes.

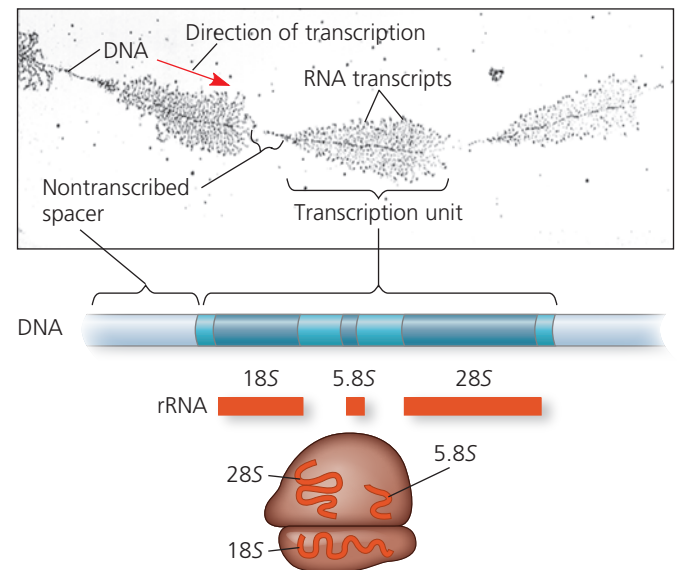
In Concept 20.5, we'll consider the evolution of these two globin gene families as we explore how arrangements of genes provide insight into the evolution of genomes. We'll also examine some processes that have shaped the genomes of different species over evolutionary time.

CONCEPT CHECK 20.4

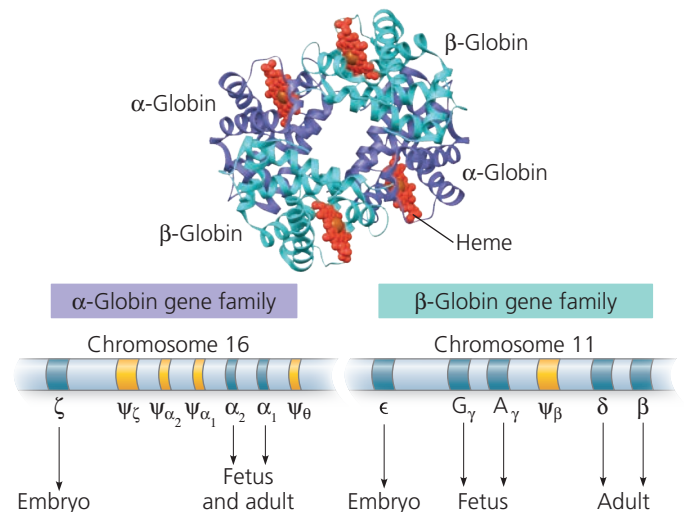
1. What is simple sequence DNA, and where is it located in a chromosome?
2. **VISUAL SKILLS** > Which of the three mechanisms described in Figures 20.8 and 20.9 result(s) in a copy remaining at the original site as well as a copy appearing in a new location?
3. Contrast the organizations of the rRNA gene family and the globin gene families. For each, explain how the existence of a family of genes benefits the organism.
4. **MAKE CONNECTIONS** > Assign each DNA segment at the top of Figure 18.8 to a sector in the pie chart in Figure 20.6.

For suggested answers, see Appendix A.

Figure 20.10 Gene families.



(a) Part of the ribosomal RNA gene family. The TEM at the top shows three of the hundreds of copies of rRNA transcription units in the rRNA gene family of a salamander genome. Each "feather" corresponds to a single unit being transcribed by about 100 molecules of RNA polymerase (dark dots along the DNA), moving left to right (red arrow). The growing RNA transcripts extend from the DNA, accounting for the feather-like appearance. In the diagram of a transcription unit below the TEM, the genes for three types of rRNA (darker blue) are adjacent to regions that are transcribed but later removed (medium blue). A single transcript is processed to yield one of each of the three rRNAs (red), key components of the ribosome.



(b) The human α -globin and β -globin gene families. Adult hemoglobin is composed of two α -globin and two β -globin polypeptide subunits, as shown in the molecular model. The genes (darker blue) encoding α - and β -globins are found in two families, organized as shown here. The noncoding DNA (light blue) separating the functional genes within each family includes pseudogenes (ψ ; gold), versions of the functional genes that no longer encode functional polypeptides. Genes and pseudogenes are named with Greek letters, as you have seen previously for the α - and β -globins. Some genes are expressed only in the embryo or fetus.

VISUAL SKILLS > In the TEM at the top of part (a), how could you determine the direction of transcription if it weren't indicated by the red arrow?

CONCEPT 20.5

Duplication, rearrangement, and mutation of DNA contribute to genome evolution

EVOLUTION Now that we have explored the makeup of the human genome, let's see what its composition reveals about how the genome evolved. The basis of change at the genomic level is mutation, which underlies much of genome evolution. It seems likely that the earliest forms of life had a minimal number of genes—those necessary for survival and reproduction. If this were indeed the case, one aspect of evolution must have been an increase in the size of the genome, with the extra genetic material providing the raw material for gene diversification. In this section, we'll first describe how extra copies of all or part of a genome can arise and then consider subsequent processes that can lead to the evolution of proteins (or RNA products) with slightly different or entirely new functions.

Duplication of Entire Chromosome Sets

An accident in meiosis, such as failure to separate homologs during meiosis I, can result in one or more extra sets of chromosomes, a condition known as polyploidy (see Concept 15.4). Although such accidents would most often be lethal, in rare cases they could facilitate the evolution of genes. In a polyploid organism, one set of genes can provide essential functions for the organism. The genes in the one or more extra sets can diverge by accumulating mutations; these variations may persist if the organism carrying them survives and reproduces. In this way, genes with novel functions can evolve. As long as one copy of an essential gene is expressed, the divergence of another copy can lead to its encoded protein acting in a novel way, thereby changing the organism's phenotype.

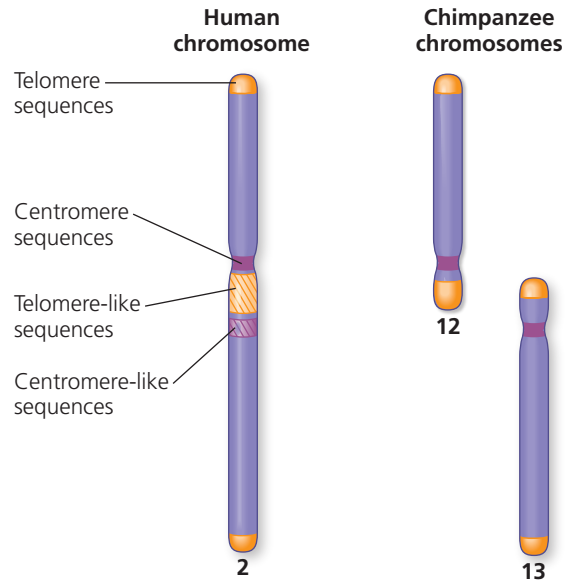
The outcome of this accumulation of mutations may eventually be the branching off of a new species. While polyploidy is rare among animals, it is relatively common among plants, especially flowering plants. Some botanists estimate that as many as 80% of the plant species that are alive today show evidence of polyploidy having occurred among their ancestral species. You'll learn more about how polyploidy leads to plant speciation in Concept 24.2.

Alterations of Chromosome Structure

With the recent explosion in genomic sequence information, we can now compare the chromosomal organizations of many different species in detail. This information allows us to make inferences about the evolutionary processes that shape chromosomes and may drive speciation. For example, scientists have long known that sometime in the

▼ Figure 20.11 Human and chimpanzee chromosomes.

The positions of telomere-like and centromere-like sequences on human chromosome 2 (left) match those of telomeres on chimpanzee chromosomes 12 and 13 and the centromere on chimpanzee chromosome 13 (right). This suggests that chromosomes 12 and 13 in a human ancestor fused end to end to form human chromosome 2. The centromere from ancestral chromosome 12 remained functional on human chromosome 2, while the one from ancestral chromosome 13 did not.

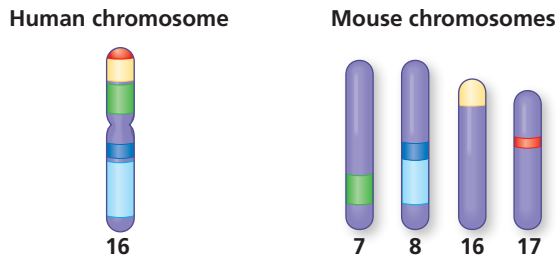


last 6 million years, when the ancestors of humans and chimpanzees diverged as species, the fusion of two ancestral chromosomes in the human line led to different haploid numbers for humans ($n = 23$) and chimpanzees ($n = 24$). The banding patterns in stained chromosomes suggested that the ancestral versions of current chimpanzee chromosomes 12 and 13 fused end to end, forming chromosome 2 in an ancestor of the human lineage. Sequencing and analysis of human chromosome 2 during the Human Genome Project provided very strong supporting evidence for the model we have just described (**Figure 20.11**).

In another study of broader scope, researchers compared the DNA sequence of each human chromosome with the whole-genome sequence of the mouse (**Figure 20.12**). One part of their study showed that large blocks of genes on human chromosome 16 are found on four mouse chromosomes, indicating that the genes in each block stayed together in both the mouse and the human lineages during their divergent evolution from a common ancestor.

Performing the same comparison of chromosomes of humans and six other mammalian species allowed the researchers to reconstruct the evolutionary history of chromosomal rearrangements in these eight species. They found many duplications and inversions of large portions of chromosomes, the result of errors during meiotic recombination in which the DNA was broken and rejoined incorrectly. The rate of these events seems to have begun accelerating about 100 million years ago, around 35 million years before large

▼ **Figure 20.12 Human and mouse chromosomes.** Here, we can see that DNA sequences very similar to large blocks of human chromosome 16 (colored areas in this diagram) are found on mouse chromosomes 7, 8, 16, and 17. This finding suggests that the DNA sequence in each block has stayed together in the mouse and human lineages since the time they diverged from a common ancestor.



dinosaurs became extinct and the number of mammalian species began rapidly increasing. The apparent coincidence is interesting because chromosomal rearrangements are thought to contribute to the generation of new species. Although two individuals with different arrangements could still mate and produce offspring, the offspring would have two nonequivalent sets of chromosomes, making meiosis inefficient or even impossible. Thus, chromosomal rearrangements would lead to two populations that could not successfully mate with each other, a step on the way to their becoming two separate species. (You'll learn more about this in Concept 24.2.)

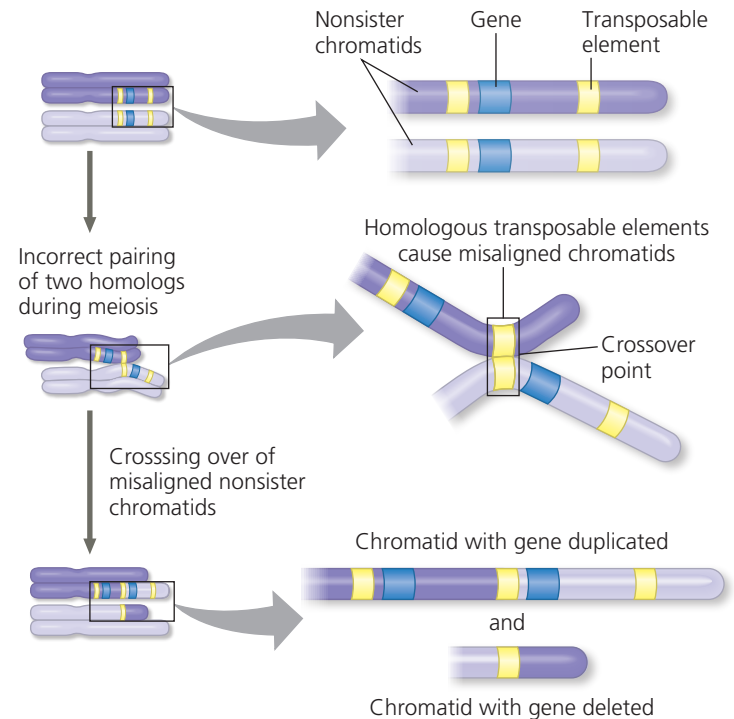
The same study also unearthed a pattern with medical relevance. Analysis of the chromosomal breakage points associated with the rearrangements showed that specific sites were used over and over again. A number of these recombination “hot spots” correspond to locations of chromosomal rearrangements within the human genome that are associated with congenital diseases (see Concept 15.4).

Duplication and Divergence of Gene-Sized Regions of DNA

Errors during meiosis can also lead to the duplication of chromosomal regions that are smaller than the ones we've just discussed, including segments the length of individual genes. Unequal crossing over during prophase I of meiosis, for instance, can result in one chromosome with a deletion and another with a duplication of a particular gene. Transposable elements can provide homologous sites where nonsister chromatids can cross over, even when other chromatid regions are not correctly aligned (**Figure 20.13**).

Also, slippage can occur during DNA replication, such that the template shifts with respect to the new complementary strand, and a part of the template strand is either skipped by the replication machinery or used twice as a template. As a result, a segment of DNA is deleted or duplicated. It is easy to imagine how such errors could occur in regions of repeats. The variable number of repeated units of simple sequence

▼ **Figure 20.13 Gene duplication due to unequal crossing over.** One mechanism by which a gene (or other DNA segment) can be duplicated is recombination during meiosis between copies of a transposable element (yellow) flanking the gene (blue). Such recombination between misaligned nonsister chromatids of homologous chromosomes produces one chromatid with two copies of the gene and one chromatid with no copy. (Genes and transposable elements are shown only in the region of interest.)



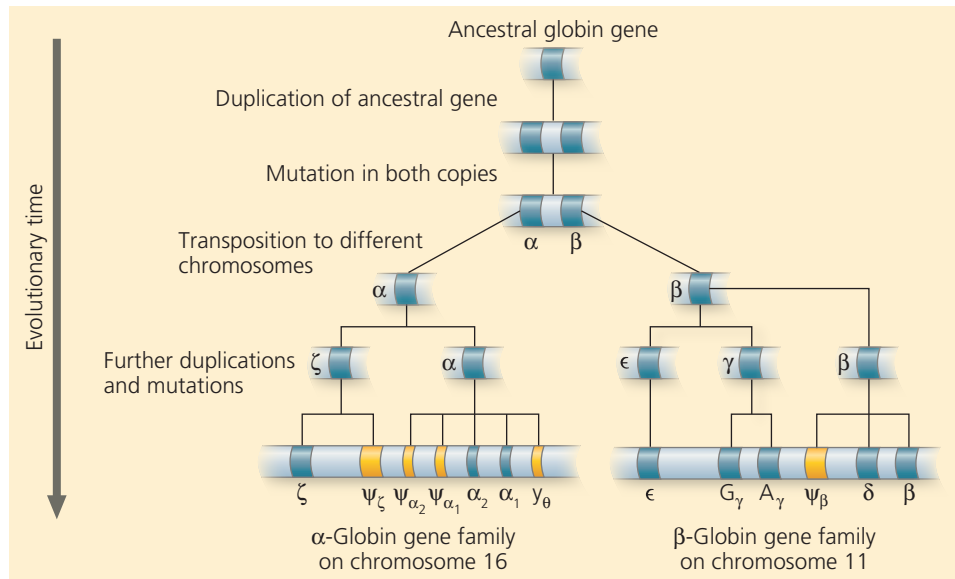
MAKE CONNECTIONS ► Examine how crossing over occurs in Figure 13.9. In the middle panel above, draw a line through the portions that result in the upper chromatid in the bottom panel. Use a different color to do the same for the other chromatid.

DNA at a given site, used for STR analysis, is probably due to errors like these. Evidence that unequal crossing over and template slippage during DNA replication lead to duplication of genes is found in the existence of multigene families, such as the globin family.

Evolution of Genes with Related Functions: The Human Globin Genes

In Figure 20.10b, you saw the organization of the α -globin and β -globin gene families as they exist in the human genome today. Now, let's consider how events such as duplications can lead to the evolution of genes with related functions like the globin genes. A comparison of gene sequences within a multigene family can suggest the order in which the genes arose. Re-creating the evolutionary history of the globin genes using this approach indicates that they all evolved from one common ancestral globin gene that underwent duplication and divergence into the α -globin and β -globin ancestral genes about 450–500 million years ago. Each of these genes was later duplicated several times, and the copies then diverged from each other in sequence, yielding the

Figure 20.14 A proposed model for the sequence of events in the evolution of the human α -globin and β -globin gene families from a single ancestral globin gene.



? The gold elements are pseudogenes. Explain how they could have arisen after gene duplication.

current family members (Figure 20.14). In fact, the common ancestral globin gene also gave rise to the oxygen-binding muscle protein myoglobin and to the plant protein leghemoglobin. The latter two proteins function as monomers, and their genes are included in a “globin superfamily.”

After the duplication events, the differences between the genes in the globin families undoubtedly arose from mutations that accumulated in the gene copies over many generations. The current model is that the necessary function provided by an α -globin protein, for example, was fulfilled by one gene, while other copies of the α -globin gene accumulated random mutations. Many mutations may have had an adverse effect on the organism, and others may have had no effect. However, a few mutations must have altered the function of the protein product in a way that benefitted the organism at a particular life stage without substantially changing the protein’s oxygen-carrying function. Presumably, natural selection acted on these altered genes, maintaining them in the population.

In the **Scientific Skills Exercise**, you can compare amino acid sequences of the globin family proteins and see how such comparisons were used to generate the model for globin gene evolution shown in Figure 20.14. The existence of several pseudogenes among the functional globin genes provides additional evidence for this model: Random mutations in these “genes” over evolutionary time have destroyed their function.

Evolution of Genes with Novel Functions

In the evolution of the globin gene families, gene duplication and subsequent divergence produced family members whose protein products performed functions similar to each

other (oxygen transport). However, an alternative scenario is that one copy of a duplicated gene can undergo alterations that lead to a completely new function for the protein product. The genes for lysozyme and α -lactalbumin are a good example.

Lysozyme is an enzyme that helps protect animals against bacterial infection by hydrolyzing bacterial cell walls (see Visualizing Figure 5.16); α -lactalbumin is a nonenzymatic protein that plays a role in milk production in mammals. The two proteins are quite similar in their amino acid sequences and three-dimensional structures (Figure 20.15). Both genes are found in mammals, but only the lysozyme gene is present in birds. These findings suggest that at some time after the lineages leading to mammals and birds had separated, the

lysozyme gene was duplicated in the mammalian lineage but not in the avian lineage. Subsequently, one copy of the duplicated lysozyme gene evolved into a gene encoding α -lactalbumin, a protein with a completely new function associated with a key characteristic of mammals—milk production. In a recent study, evolutionary biologists searched vertebrate genomes for genes with similar sequences. There appear to be at least eight members of the lysozyme family, distributed widely among mammalian species. The functions of all the encoded gene products are not yet known, but it will be exciting to discover whether they are as different as the functions of lysozyme and α -lactalbumin.

Besides the duplication and divergence of whole genes, rearrangement of existing DNA sequences within genes has also contributed to genome evolution. The presence of introns may have promoted the evolution of new proteins by facilitating the duplication or shuffling of exons, as we’ll discuss next.

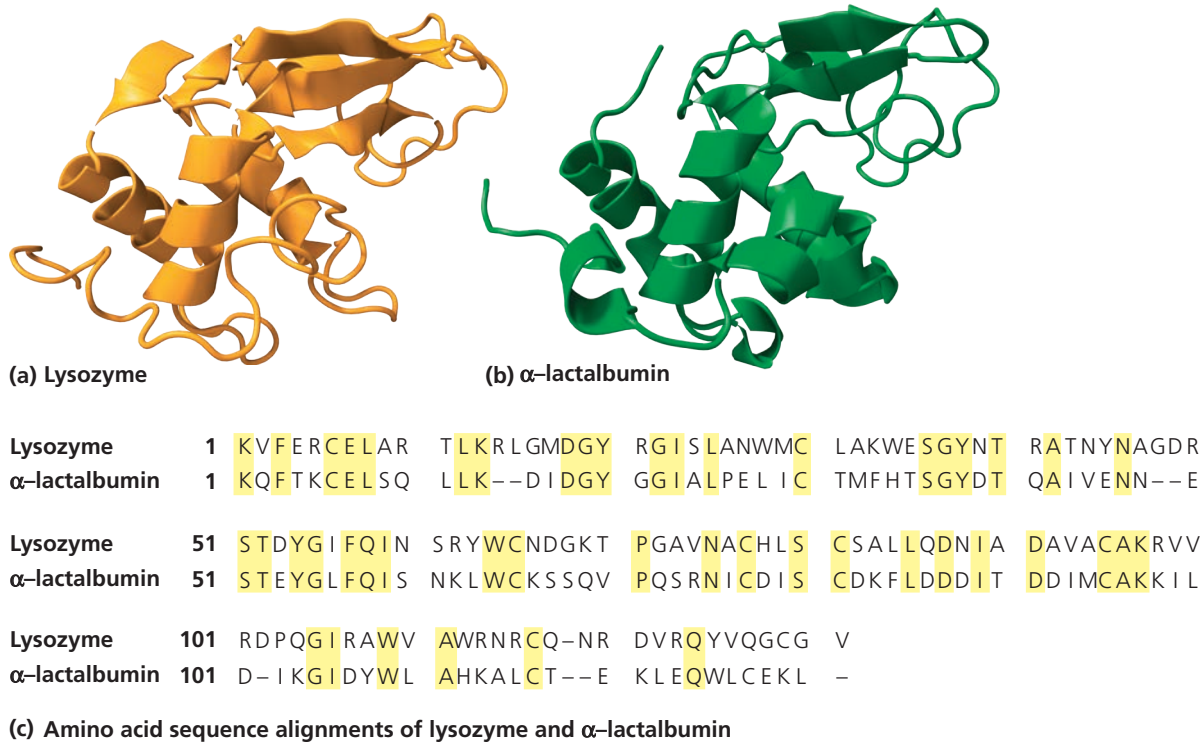
Rearrangements of Parts of Genes: Exon Duplication and Exon Shuffling

Recall from Concept 17.3 that an exon often codes for a protein domain, a distinct structural and functional region of a protein molecule. We’ve already seen that unequal crossing over during meiosis can lead to duplication of a gene on one chromosome and its loss from the homologous chromosome (see Figure 20.13). By a similar process, a particular exon within a gene could be duplicated on one chromosome and deleted from the other. The gene with the duplicated exon would code for a protein containing a second copy

▼ **Figure 20.15 Comparison of lysozyme and α -lactalbumin proteins.**

Computer-generated ribbon models of the similar structures of (a) lysozyme and (b) α -lactalbumin are shown, along with (c) a comparison of the amino acid sequences of the two proteins. The amino acids are arranged in groups of ten for ease of reading, and single-letter amino acid codes are used (see Figure 5.14). Identical amino acids are highlighted in yellow, and dashes indicate gaps in one sequence that have been introduced by the software to optimize the alignment.

MAKE CONNECTIONS ► Even though two amino acids are not identical, they may be structurally and chemically similar and therefore behave similarly. Using Figure 5.14 as a reference, examine the nonidentical amino acids in positions 1–30 and note cases where the amino acids in the two sequences are similarly acidic or basic.

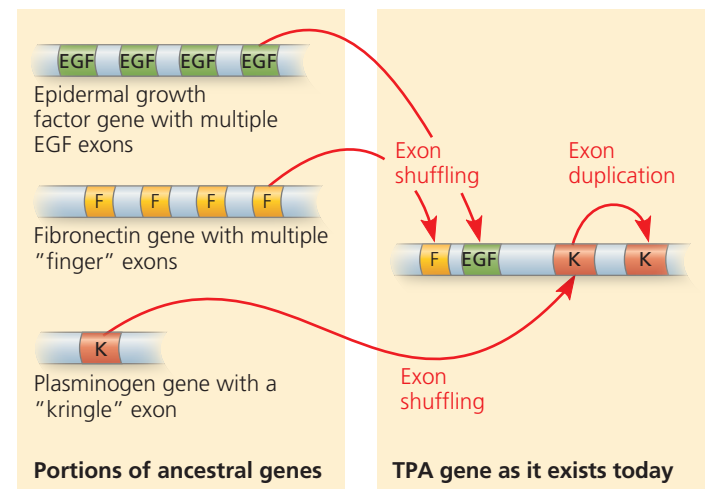


of the encoded domain. This change in the protein's structure might augment its function by increasing its stability, enhancing its ability to bind a particular ligand, or altering some other property. Quite a few protein-coding genes have multiple copies of related exons, which presumably arose by duplication and then diverged. The gene encoding the extracellular matrix protein collagen is a good example. Collagen is a structural protein (see Figure 5.18) with a highly repetitive amino acid sequence, which reflects the repetitive pattern of exons in the collagen gene.

As an alternative possibility, we can imagine the occasional mixing and matching of different exons either within a gene or between two different (nonallelic) genes owing to errors in meiotic recombination. This process, termed *exon shuffling*, could lead to new proteins with novel combinations of functions. As an example, let's consider the gene for tissue plasminogen activator (TPA). The TPA protein is an extracellular protein that helps control blood clotting. It has four domains of three types, each encoded by an exon, and one of those exons is present in two copies. Because each type of exon is also found in other proteins, the current version of the gene for TPA is thought to have arisen by several instances of exon shuffling during errors in meiotic recombination and subsequent duplication (Figure 20.16).

▼ **Figure 20.16 Evolution of a new gene by exon shuffling.**

Meiotic errors could have moved exons, each encoding a particular domain, from ancestral forms of the genes for epidermal growth factor, fibronectin, and plasminogen (left) into the evolving gene for tissue plasminogen activator, TPA (right). Subsequent duplication of the "kringle" exon (K) from the plasminogen gene after its movement into the TPA gene could account for the two copies of this exon in the TPA gene existing today.



VISUAL SKILLS ► Looking at Figure 20.13, describe the steps by which transposable elements within introns might have facilitated the exon shuffling shown here.

SCIENTIFIC SKILLS EXERCISE

Reading an Amino Acid Sequence Identity Table

How Have Amino Acid Sequences of Human Globin Genes Diverged During Their Evolution?

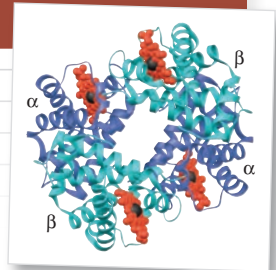
To build a model of the evolutionary history of the globin genes (see Figure 20.14), researchers compared the amino acid sequences of the polypeptides they encode. In this exercise, you will analyze comparisons of the amino acid sequences of the globin polypeptides to shed light on their evolutionary relationships.

How the Experiment Was Done Scientists obtained the DNA sequences for each of the eight globin genes and “translated” them into amino acid sequences. They then used a computer program to align the sequences (with dashes indicating gaps in one sequence) and calculate a percent identity value for each pair of globins. The percent identity reflects the number of positions with identical amino acids relative to the total number of amino acids in a globin polypeptide. The data were displayed in a table to show the pairwise comparisons.

Data from the Experiment The following table shows an example of a pairwise alignment—that of the α_1 -globin (alpha-1 globin) and ζ -globin (zeta globin) amino acid sequences—using the standard single-letter symbols for amino acids. To the left of each line of amino acid sequence is the number of the first amino acid in that line. The percent identity value for the α_1 - and ζ -globin amino acid sequences was calculated by counting the number of matching amino acids

Globin	Alignment of Globin Amino Acid Sequences
α_1	1 MVLSPADKTNVKAAGKVGAGHAGEYGAEAL
ζ	1 MSLTKTERTIIIVSMWAKISTQADTIGTETL
α_1	31 ERMFLSFPTTKTYFPHFDLSH-GSAQVKGH
ζ	31 ERLFLSHPQTKTYFPHFDL-HPGSAQLRAH
α_1	61 GKKVADALTNVAHVDDMPNALSALSDLHA
ζ	61 GSKVVAAVGDAVKSIDDIGGALSCLSELHA
α_1	91 HKLRVDPVNFKLLSHCLLVTLAAHLPAEFT
ζ	91 YILRVDPVNFKLLSHCLLVTLAARFPADFT
α_1	121 PAVHASLDKFLASVSTVLTSKYR
ζ	121 AEAAAWDKFLSVVSSVLTEKYR

(86, highlighted in yellow), dividing by the total number of amino acid positions (143), and then multiplying by 100. This resulted in a 60% identity value for the α_1 - ζ pair, as shown in the amino acid identity table at the bottom of the page. The values for other globin pairs were calculated in the same way.



Hemoglobin

INTERPRET THE DATA

- Note that in the amino acid identity table, the data are arranged so each globin pair can be compared. (a) Some cells in the table have dashed lines. Given the pairs that are being compared for these cells, what percent identity value is implied by the dashed lines? (b) Notice that the cells in the lower left half of the table are blank. Using the information already provided in the table, fill in the missing values. Why does it make sense that these cells were left blank in the table?
- The earlier that two genes arose from a duplicated gene, the more their nucleotide sequences can have diverged, which may result in amino acid differences in the protein products. (a) Based on that premise, identify which two genes are most divergent from each other. What is the percent amino acid identity between their polypeptides? (b) Using the same approach, identify which two globin genes are the most recently duplicated. What is the percent identity between them?
- The model of globin gene evolution shown in Figure 20.14 suggests that an ancestral gene duplicated and mutated to become α - and β -globin genes, and then each one was further duplicated and mutated. What features of the data set support the model?
- Make an ordered list of all the percent identity values from the table, starting with 100% at the top. Next to each number write the globin pair(s) with that percent identity value. Use one color for the globins from the α family and a different color for the globins from the β family. (a) Compare the order of pairs on your list with their positions in the model shown in Figure 20.14. Does the order of pairs describe the same relative “closeness” of globin family members seen in the model? (b) Compare the percent identity values for pairs within the α or β group to the values for between-group pairs.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Further Reading R. C. Hardison, Globin genes on the move, *Journal of Biology* 7:35.1–35.5 (2008).

Amino Acid Identity Table									
α Family					β Family				
		α_1 (alpha 1)	α_2 (alpha 2)	ζ (zeta)	β (beta)	δ (delta)	ϵ (epsilon)	A_γ (gamma A)	G_γ (gamma G)
α Family	α_1	-----	100	60	45	44	39	42	42
	α_2		-----	60	45	44	39	42	42
	ζ			-----	38	40	41	41	41
β Family	β				-----	93	76	73	73
	δ					-----	73	71	72
	ϵ						-----	80	80
	A_γ							-----	99
	G_γ								-----

Compiled using data from the National Center for Biotechnology Information (NCBI).

How Transposable Elements Contribute to Genome Evolution

The persistence of transposable elements as a large fraction of some eukaryotic genomes is consistent with the idea that they play an important role in shaping a genome over evolutionary time. These elements can contribute to the evolution of the genome in several ways. They can promote recombination, disrupt cellular genes or control elements, and carry entire genes or individual exons to new locations.

Transposable elements of similar sequence scattered throughout the genome facilitate recombination between different (nonhomologous) chromosomes by providing homologous regions for crossing over (see Figure 20.13). Most such recombination events are probably detrimental, causing chromosomal translocations and other changes in the genome that may be lethal to the organism. But over the course of evolutionary time, an occasional recombination event of this sort may be advantageous to the organism. (For the change to be heritable, of course, it must happen in a cell that will give rise to a gamete.)

The movement of a transposable element can have a variety of consequences. For instance, a transposable element that “jumps” into a protein-coding sequence will prevent the production of a normal transcript of the gene. (Introns provide a sort of “safety zone” that does not affect the transcript because the transposable element will be spliced out.) If a transposable element inserts within a regulatory sequence, the transposition may lead to increased or decreased production of one or more proteins. Transposition caused both types of effects on the genes coding for pigment-synthesizing enzymes in McClintock’s corn kernels. Again, while such changes are usually harmful, in the long run some may provide a survival advantage. A possible example was mentioned earlier: At least some of the *Alu* transposable elements in the human genome are known to produce RNAs that regulate expression of human genes.

During transposition, a transposable element may carry along a gene or even a group of genes to a new position in the genome. This mechanism probably accounts for the location of the α -globin and β -globin gene families on different human chromosomes, as well as the dispersion of the genes of certain other gene families. By a similar tag-along process, an exon from one gene may be inserted into another gene in a mechanism similar to that of exon shuffling during recombination. For example, an exon may be inserted by transposition into the intron of a protein-coding gene. If the inserted exon is retained in the RNA transcript during RNA splicing, the protein that is synthesized will have an additional domain, which may confer a new function on the protein.

Most often, the processes discussed in this section produce harmful effects, which may be lethal, or have no effect at all. In a few cases, however, small heritable changes that are beneficial may occur. Over many generations, the resulting

genetic diversity provides valuable raw material for natural selection. Diversification of genes and their products is an important factor in the evolution of new species. Thus, the accumulation of changes in the genome of each species provides a record of its evolutionary history. To read this record, we must be able to identify genomic changes. Comparing the genomes of different species allows us to do that, increasing our understanding of how genomes evolve. You will learn more about these topics next.

CONCEPT CHECK 20.5

1. Describe three examples of errors in cellular processes that lead to DNA duplications.
2. Explain how multiple exons might have arisen in the ancestral EGF and fibronectin genes shown on the left side of Figure 20.16.
3. What are three ways that transposable elements are thought to contribute to genome evolution?
4. **WHAT IF? >** In 2005, Icelandic scientists reported finding a large chromosomal inversion present in 20% of northern Europeans, and they noted that Icelandic women with this inversion had significantly more children than women without it. What would you expect to happen to the frequency of this inversion in the Icelandic population in future generations?

For suggested answers, see Appendix A.

CONCEPT 20.6

Comparing genome sequences provides clues to evolution and development

EVOLUTION One researcher has likened the current state of biology to the Age of Exploration in the 1400s, which occurred soon after major improvements in navigation and ship design. In the last 30 years, we have seen rapid advances in genome sequencing and data collection, new techniques for assessing gene activity across the whole genome, and refined approaches for understanding how genes and their products work together in complex systems. In the field of biology, we are truly poised on the brink of a new world.

Comparisons of genome sequences from different species reveal a lot about the evolutionary history of life, from very ancient to more recent. Similarly, comparative studies of the genetic programs that direct embryonic development in different species are beginning to clarify the mechanisms that generated the great diversity of life-forms present today. In this final section of the chapter, we will discuss what has been learned from these two approaches.

Comparing Genomes

The more similar in sequence the genes and genomes of two species are, the more closely related those species are in their

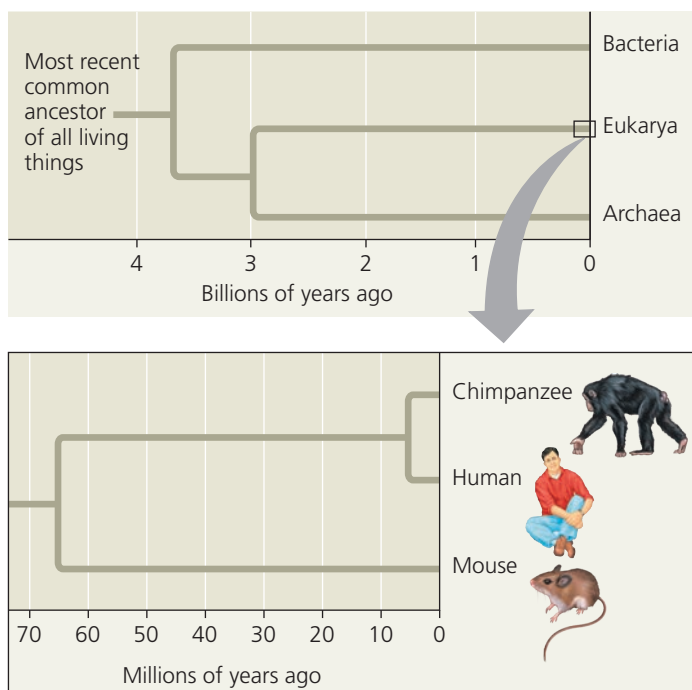
evolutionary history—because not enough time has passed for mutations and other changes to accumulate. Comparing genomes of closely related species sheds light on more recent evolutionary events, whereas comparing genomes of very distantly related species helps us understand ancient evolutionary history. In either case, learning about characteristics that are shared or divergent between groups enhances our picture of the evolution of organisms and biological processes. As you learned in Concept 1.2, the evolutionary relationships between species can be represented by a diagram in the form of a tree, where each branch point marks the divergence of two lineages. **Figure 20.17** shows the evolutionary relationships of some groups and species we will discuss.

Comparing Distantly Related Species

Determining which genes have remained similar—that is, are *highly conserved*—in distantly related species can help clarify evolutionary relationships among species that diverged from each other long ago. Indeed, comparisons of the specific gene sequences of bacteria, archaea, and eukaryotes indicate that these three groups diverged between 2 and 4 billion years ago and strongly support the theory that they are the fundamental domains of life (see Figure 20.17).

In addition to their value in evolutionary biology, comparative genomic studies confirm the relevance of research on model organisms to our understanding of biology in general and human biology in particular. Very ancient genes

▼ **Figure 20.17 Evolutionary relationships of the three domains of life.** The tree diagram at the top shows the ancient divergence of bacteria, archaea, and eukaryotes. A portion of the eukaryote lineage is expanded in the inset to show the more recent divergence of three mammalian species discussed in this chapter.



can still be surprisingly similar in disparate species. A 2015 study tested the ability of the human version of each of 414 important yeast genes to function equivalently in yeast cells. Remarkably, the researchers concluded that 47% of these yeast genes could be replaced by the human gene. This striking result underscores the common origin of yeasts and humans—two distantly related species.

Comparing Closely Related Species

The genomes of two closely related species are likely to be organized similarly because of their relatively recent divergence. Their long shared history also means that only a small number of gene differences are found when their genomes are compared. These genetic differences can thus be more easily correlated with phenotypic differences between the two species. An exciting application of this type of analysis is seen as researchers compare the human genome with the genomes of the chimpanzee, mouse, rat, and other mammals. Identifying the genes shared by all of these species but not by nonmammals gives us clues about what it takes to make a mammal, while finding the genes shared by chimpanzees and humans but not by rodents tells us something about primates. And, of course, comparing the human genome with that of the chimpanzee helps us answer the tantalizing question we asked at the beginning of the chapter: What genomic information defines a human or a chimpanzee?

An analysis of the overall composition of the human and chimpanzee genomes, which are thought to have diverged only about 6 million years ago (see Figure 20.17), reveals some general differences. Considering single nucleotide substitutions, the two genomes differ by only 1.2%. When researchers looked at longer stretches of DNA, however, they were surprised to find a further 2.7% difference due to insertions or deletions of larger regions in the genome of one or the other species; many of the insertions were duplications or other repetitive DNA. In fact, a third of the human duplications are not present in the chimpanzee genome, and some of these duplications contain regions associated with human diseases. There are more *Alu* elements in the human genome than in the chimpanzee genome, and the latter contains many copies of a retroviral provirus (see Chapter 26) not present in humans. All of these observations provide clues to the forces that might have swept the two genomes along different paths, but we don't have a complete picture yet.

Along with chimpanzees, bonobos are the other African ape species that are the closest living relatives to humans. The sequencing of the bonobo genome, completed in 2012, revealed that in some regions, human sequences were more closely related to either chimpanzee or bonobo sequences than chimpanzee or bonobo sequences were to each other. Such a fine-grained comparison of three closely related species allows even more detail to be worked out in reconstructing their related evolutionary history.

We also don't know how the genetic differences revealed by genome sequencing might account for the distinct characteristics of each species. To discover the basis for the phenotypic differences between chimpanzees and humans, biologists are studying specific genes and types of genes that differ between the two species and comparing them with their counterparts in other mammals. This approach has revealed a number of genes that are apparently changing (evolving) faster in the human than in either the chimpanzee or the mouse. Among them are genes involved in defense against

malaria and tuberculosis as well as at least one gene that regulates brain size. When genes are classified by function, the genes that seem to be evolving the fastest are those that code for transcription factors. This discovery makes sense because transcription factors regulate gene expression and thus play a key role in orchestrating the overall genetic program.

One transcription factor whose gene shows evidence of rapid change in the human lineage is called *FOXP2* (**Figure 20.18**). Several lines of evidence suggest that the *FOXP2* gene product regulates genes that function in

▼ Figure 20.18

Inquiry What is the function of a gene (*FOXP2*) that is rapidly evolving in the human lineage?

Experiment Several lines of evidence support a role for the *FOXP2* gene in the development of speech and language in humans and of vocalization in other vertebrates. In 2005, Joseph Buxbaum and collaborators at the Mount Sinai School of Medicine and several other institutions tested the function of *FOXP2*. They used the mouse, a model organism in which genes can be easily knocked out, as a representative vertebrate that vocalizes: Mice produce ultrasonic squeaks (whistles) to communicate stress. The researchers used genetic engineering to produce mice in which one or both copies of *FOXP2* were disrupted.

Wild type: two normal copies of *FOXP2*

Heterozygote: one copy of *FOXP2* disrupted

Homozygote: both copies of *FOXP2* disrupted

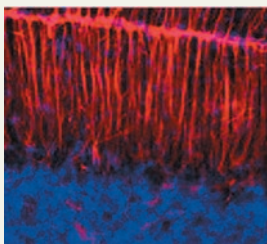
They then compared the phenotypes of these mice. Two of the characters they examined are included here: brain anatomy and vocalization.



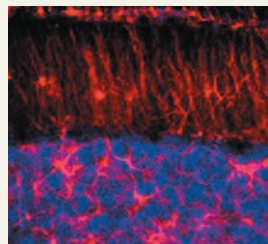
Experiment 1: Researchers cut thin sections of brain and stained them with reagents that allow visualization of brain anatomy in a UV fluorescence microscope.

Results

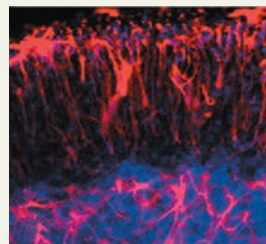
Experiment 1 Results: Disruption of both copies of *FOXP2* led to brain abnormalities in which the cells were disorganized. Phenotypic effects on the brain of heterozygotes, with one disrupted copy, were less severe. (Each color in the micrographs below reveals a different cell or tissue type.)



Wild type



Heterozygote



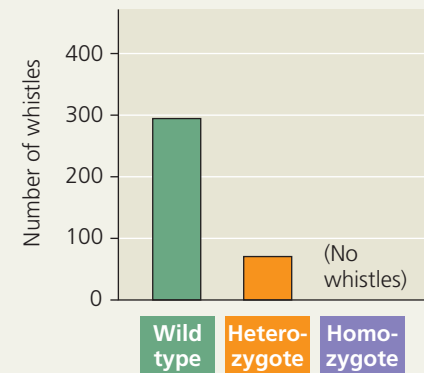
Homozygote

Conclusion *FOXP2* plays a significant role in the development of functional communication systems in mice. The results augment evidence from studies of birds and humans, supporting the hypothesis that *FOXP2* may act similarly in diverse organisms.

Data from W. Shu et al., Altered ultrasonic vocalization in mice with a disruption in the *Foxp2* gene, *Proceedings of the National Academy of Sciences USA* 102:9643–9648 (2005).

Experiment 2: To induce stress, researchers separated each newborn mouse pup from its mother and recorded the number of ultrasonic whistles produced by the pup.

Experiment 2 Results: Disruption of both copies of *FOXP2* led to an absence of ultrasonic vocalization in response to stress. The effect on vocalization in the heterozygote was also extreme.



WHAT IF? ► Since the results support a role for mouse *FOXP2* in vocalization, you might wonder whether the human *FOXP2* protein is a key regulator of speech. If you were given the amino acid sequences of wild-type and mutant human *FOXP2* proteins and the wild-type chimpanzee *FOXP2* protein, how would you investigate this question? What further clues could you obtain by comparing these sequences to that of the mouse *FOXP2* protein?

vocalization in vertebrates. First, mutations in this gene can produce severe speech and language impairment in humans. Moreover, the *FOXP2* gene is expressed in the brains of zebra finches and canaries at the time when these songbirds are learning their songs. But perhaps the strongest evidence comes from a “knockout” experiment in which researchers disrupted the *FOXP2* gene in mice and analyzed the resulting phenotype (see Figure 20.18). The homozygous mutant mice had malformed brains and failed to emit normal ultrasonic vocalizations, and mice with one faulty copy of the gene also showed significant problems with vocalization. These results support the idea that the *FOXP2* gene product turns on genes involved in vocalization.

Expanding on this analysis, another research group more recently replaced the *FOXP2* gene in mice with a “humanized” copy coding for the human versions of two amino acids that differ between human and chimpanzee; these are the changes potentially responsible for a human’s ability to speak. Although the mice were generally healthy, they had subtly different vocalizations and showed changes in brain cells in circuits associated with speech in human brains.

In 2010, the Neanderthal genome was sequenced from a very small amount of preserved genomic DNA, and a high-quality sequence was completed in 2014. Neanderthals (*Homo neanderthalensis*) are members of the same genus to which humans (*Homo sapiens*) belong (see Concept 34.7). A reconstruction of their evolutionary history based on genomic comparisons between the two species suggests that some groups of humans and Neanderthals co-existed and interbred for a period of time before Neanderthals went extinct about 30,000 years ago. While Neanderthals have sometimes been portrayed as primitive beings that could only grunt, their *FOXP2* gene sequence encodes a protein identical to that of humans. This suggests that Neanderthals may have been capable of speech of some type and, along with other observed genetic similarities, forces us to reevaluate our image of our recent extinct relatives.

The *FOXP2* story is an excellent example of how different approaches can complement each other in uncovering biological phenomena of widespread importance. The *FOXP2* experiments used mice as a model for humans because it would be unethical (as well as impractical) to carry out such experiments in humans. Mice and humans, which diverged about 65.5 million years ago (see Figure 20.17), share about 85% of their genes. This genetic similarity can be exploited in studying human genetic disorders. If researchers know the organ or tissue that is affected by a particular genetic disorder, they can look for genes that are expressed in these locations in mice.

Even though more distantly related to humans, fruit flies have also been a useful model species for study of such human disorders as Parkinson’s disease and alcoholism, while nematodes (soil worms) have yielded a wealth of information about aging. Further research efforts are under way to extend

genomic studies to many more species, including neglected species from diverse branches of the tree of life. These studies will advance our understanding of evolution, of course, as well as all aspects of biology, from human health to ecology.

Comparing Genomes Within a Species

Another exciting consequence of our ability to analyze genomes is our growing understanding of the spectrum of genetic variation in humans. Because the history of the human species is so short—probably about 200,000 years—the amount of DNA variation among humans is small compared to that of many other species. Much of our diversity seems to be in the form of single nucleotide polymorphisms (SNPs). SNPs are single base-pair sites where genetic variation is found in at least 1% of the population (see Concept 19.2); they are usually detected by DNA sequencing. In the human genome, SNPs occur on average about once in 100–300 base pairs. Scientists have already identified the locations of several million SNP sites in the human genome and continue to find additional SNPs. These are stored in databases around the world, one of which is run by the National Center for Biotechnology Information (NCBI) and can be accessed at <http://www.ncbi.nlm.nih.gov/SNP/>.

In the course of this search, they have also found other variations—including chromosomal regions with inversions, deletions, and duplications. The most surprising discovery has been the widespread occurrence of *copy-number variants* (CNVs), loci where some individuals have one or multiple copies of a particular gene or genetic region rather than the standard two copies (one on each homolog). CNVs result from regions of the genome being duplicated or deleted inconsistently within the population. One study of 40 people found more than 8,000 CNVs involving 13% of the genes in the genome, and these CNVs probably represent just a small subset of the total. Since these variants encompass much longer stretches of DNA than the single nucleotides of SNPs, CNVs are more likely to have phenotypic consequences and to play a role in complex diseases and disorders. At the very least, the high incidence of copy-number variation blurs the meaning of the phrase “a normal human genome.”

Copy-number variants, SNPs, and variations in repetitive DNA such as short tandem repeats (STRs) are useful genetic markers for studying human evolution. In one study, the genomes of two Africans from different communities were sequenced: Archbishop Desmond Tutu, the South African civil rights advocate and a member of the Bantu tribe, the majority population in southern Africa, and !Gubi, a hunter-gatherer from the Khoisan community in Namibia, a minority African population that is probably the human group with the oldest known lineage. The comparison revealed many differences, as you might expect. The analysis was then broadened to compare the protein-coding regions of !Gubi’s genome with those of three other

Khoisan community members (self-identified Bushmen) living nearby. Remarkably, the four African genomes differed more from each other than a European would from an Asian. These data highlight the extensive diversity among African genomes. Extending this approach will help us answer important questions about the differences between human populations and the migratory routes of human populations throughout history.

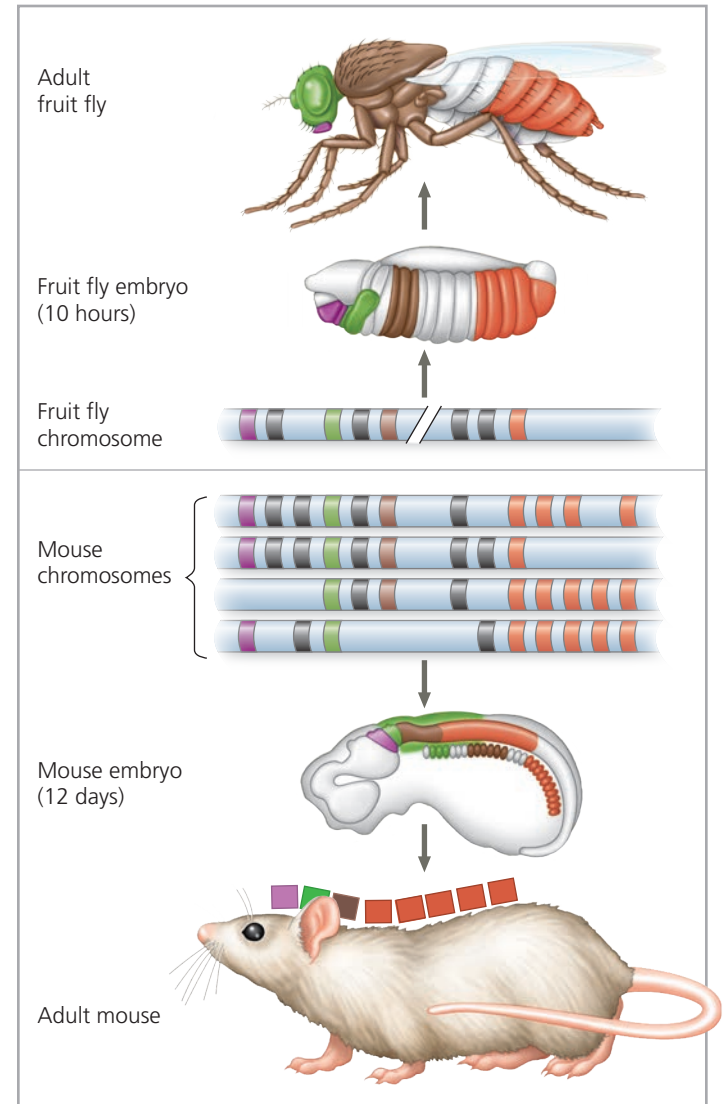
Widespread Conservation of Developmental Genes Among Animals

Biologists in the field of evolutionary developmental biology, or **evo-devo** as it is often called, compare developmental processes of different multicellular organisms. Their aim is to understand how these processes have evolved and how changes in them can modify existing organismal features or lead to new ones. With the advent of molecular techniques and the recent flood of genomic information, we are beginning to realize that the genomes of related species with strikingly different forms may have only minor differences in gene sequence or, perhaps more importantly, in gene regulation. Discovering the molecular basis of these differences in turn helps us understand the origins of the myriad diverse forms that cohabit this planet, thus informing our study of the evolution of life.

In Concept 18.4, you learned about the homeotic genes in *Drosophila melanogaster* (see Figure 18.20), which encode transcription factors that regulate gene expression and specify the identity of body segments in the fruit fly. Molecular analysis of the homeotic genes in *Drosophila* has shown that they all include a 180-nucleotide sequence called a **homeobox**, which codes for a 60-amino-acid *homeodomain* in the encoded proteins. An identical or very similar nucleotide sequence has been discovered in the homeotic genes of many invertebrates and vertebrates. In fact, the nucleotide sequences in humans and fruit flies are so similar that one researcher has whimsically referred to flies as “little people with wings.” The resemblance even extends to the organization of these genes: The vertebrate genes homologous to the homeotic genes of fruit flies have kept the same chromosomal arrangement (**Figure 20.19**). Homeobox-containing sequences have also been found in regulatory genes of much more distantly related eukaryotes, including plants and yeasts. From these similarities, we can deduce that the homeobox DNA sequence evolved very early in the history of life and was sufficiently valuable to organisms to have been conserved in animals and plants virtually unchanged for hundreds of millions of years.

Homeotic genes in animals were named *Hox* genes, short for homeobox-containing genes, because homeotic genes were the first genes found to have this sequence. Other homeobox-containing genes were later found that do not act as homeotic genes; that is, they do not directly control

▼ **Figure 20.19 Conservation of homeotic genes in a fruit fly and a mouse.** Homeotic genes that control the form of anterior and posterior structures of the body occur in the same linear sequence on chromosomes in *Drosophila* and mice. Each colored band on the chromosomes shown here represents a homeotic gene. In fruit flies, all homeotic genes are found on one chromosome. The mouse and other mammals have the same or similar sets of genes on four chromosomes. The color code indicates the parts of the embryos in which these genes are expressed and the adult body regions that result. All of these genes are essentially identical in flies and mice, except for those represented by black bands, which are less similar in the two animals.



the identity of body parts. However, most of these genes, in animals at least, are associated with development, suggesting their ancient and fundamental importance in that process. In *Drosophila*, for example, homeoboxes are present not only in the homeotic genes but also in the egg-polarity gene *bicoid* (see Figures 18.21 and 18.22), in several of the segmentation genes, and in a master regulatory gene for eye development.

Researchers have discovered that the homeobox-encoded homeodomain binds to DNA when the protein functions as a transcription factor. Elsewhere in the protein, domains that

are more variable interact with other transcription factors, allowing the homeodomain-containing protein to recognize specific enhancers and regulate the associated genes. Proteins with homeodomains probably regulate development by coordinating the transcription of batteries of developmental genes, switching them on or off. In embryos of *Drosophila* and other animal species, different combinations of homeobox genes are active in different parts of the embryo. This selective expression of regulatory genes, varying over time and space, is central to pattern formation.

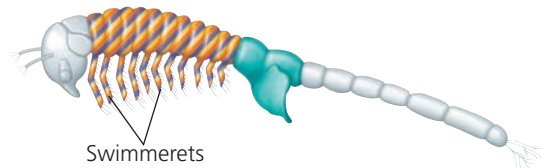
Developmental biologists have found that in addition to homeotic genes, many other genes involved in development are highly conserved from species to species. These include numerous genes encoding components of signaling pathways. The extraordinary similarity among some developmental genes in different animal species raises a question: How can the same genes be involved in the development of animals whose forms are so very different from each other?

Ongoing studies are suggesting answers to this question. In some cases, small changes in regulatory sequences of particular genes cause changes in gene expression patterns that can lead to major changes in body form. For example, the differing patterns of expression of the *Hox* genes along the body axis in a crustacean and an insect can explain the variation in number of leg-bearing segments among these closely related animals (**Figure 20.20**). In other cases, similar genes direct different developmental processes in various organisms, resulting in diverse body shapes. Several *Hox* genes, for instance, are expressed in the embryonic and larval stages of the sea urchin, a nonsegmented animal that has a body plan quite different from those of insects and mice. Sea urchin adults make the pincushion-shaped shells you may have seen on the beach; two species of live sea urchins are shown in the photo. Sea urchins are among the organisms long used in classical embryological studies (see Concept 46.2).

In this chapter of the genetics unit, you have learned how studying genomic composition and comparing the genomes of different species can illuminate the process by which genomes evolve. Furthermore, comparing developmental programs, we can see that the

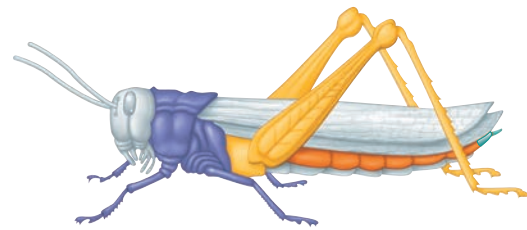
▼ Figure 20.20 Effect of differences in *Hox* gene expression in a crustacean and an insect.

Changes in the expression patterns of *Hox* genes have occurred over evolutionary time since insects diverged from a crustacean ancestor. These changes account in part for the different body plans of (a) the brine shrimp *Artemia*, a crustacean, and (b) the grasshopper, an insect. Shown here are regions of the adult body color-coded for expression of four *Hox* genes that determine the formation of particular body parts during embryonic development. Each color represents a specific *Hox* gene.



(a) Expression of four *Hox* genes in the brine shrimp *Artemia*.

Three of the *Hox* genes are expressed together in one region (indicated by stripes), specifying the identity of the segments that have swimmerets. The fourth (teal) specifies the identity of the genital segments.



(b) Expression of the grasshopper versions of the same four *Hox* genes.

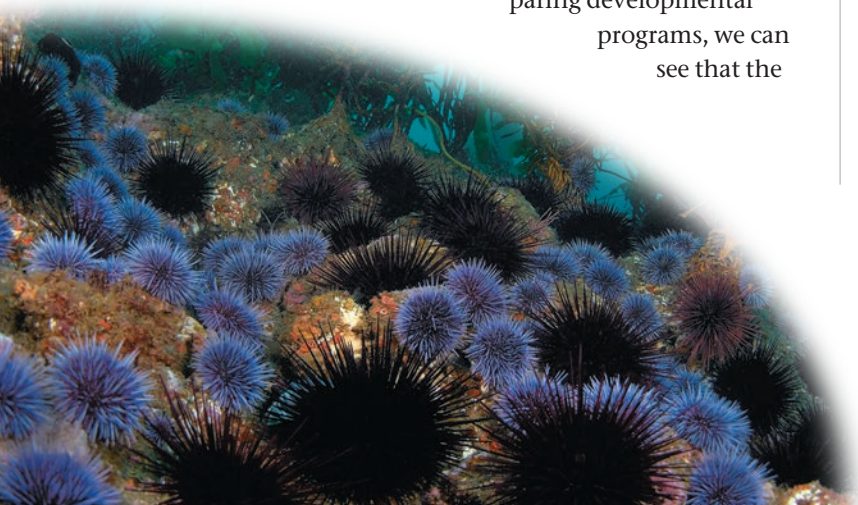
In the grasshopper, each *Hox* gene is expressed in a discrete region and specifies the identity of that region.

unity of life is reflected in the similarity of molecular and cellular mechanisms used to establish body pattern, although the genes directing development may differ among organisms. The similarities between genomes reflect the common ancestry of life on Earth. But the differences are also crucial, for they have created the huge diversity of organisms that have evolved. In the remaining chapters, we expand our perspective beyond the level of molecules, cells, and genes to explore this diversity on the organismal level.

CONCEPT CHECK 20.6

1. Would you expect the genome of the macaque (a monkey) to be more like that of a mouse or that of a human? Explain.
2. DNA sequences called homeoboxes help homeotic genes in animals direct development. Given that they are common to flies and mice, explain why these animals are so different.
3. **WHAT IF? >** There are three times as many *Alu* elements in the human genome as in the chimpanzee genome. How do you think these extra *Alu* elements arose in the human genome? Propose a role they might have played in the divergence of these two species.

For suggested answers, see Appendix A.



20 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 20.1

The Human Genome Project fostered development of faster, less expensive sequencing techniques (pp. 475–476)



- The **Human Genome Project** was largely completed in 2003, aided by major advances in sequencing technology.
- In the **whole-genome shotgun** approach, the whole genome is cut into many small, overlapping fragments that are sequenced; computer software then assembles the genome sequence.

? How did the Human Genome Project result in more rapid, less expensive DNA-sequencing technology?

CONCEPT 20.2

Scientists use bioinformatics to analyze genomes and their functions (pp. 476–480)

- Computer analysis of genome sequences aids **gene annotation**, the identification of protein-coding sequences. Methods to determine gene function include comparing sequences of newly discovered genes with those of known genes in other species and observing the effects of experimentally inactivating the genes.
- In **systems biology**, scientists use the computer-based tools of **bioinformatics** to compare genomes and study sets of genes and proteins as whole systems (**genomics** and **proteomics**). Studies include large-scale analyses of protein interactions, functional DNA elements, and genes contributing to medical conditions.

? What has been the most significant finding of the ENCODE project? Why was the project expanded to include non-human species?

CONCEPT 20.3

Genomes vary in size, number of genes, and gene density (pp. 480–482)

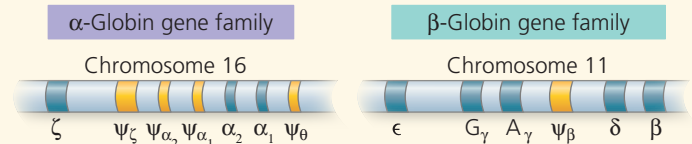
	Bacteria	Archaea	Eukarya
Genome size	Most are 1–6 Mb		Most are 10–4,000 Mb, but a few are much larger
Number of genes	1,500–7,500		Most are 5,000–45,000
Gene density	Higher than in eukaryotes		Lower than in prokaryotes (Within eukaryotes, lower density is correlated with larger genomes.)
Introns	None in protein-coding genes	Present in some genes	Present in most genes of multicellular eukaryotes, but only in some genes of unicellular eukaryotes
Other noncoding DNA	Very little		Can exist in large amounts; generally more repetitive noncoding DNA in multicellular eukaryotes

? Compare genome size, gene number, and gene density (a) in the three domains and (b) among eukaryotes.

CONCEPT 20.4

Multicellular eukaryotes have a lot of noncoding DNA and many multigene families (pp. 482–485)

- Only 1.5% of the human genome codes for proteins or gives rise to rRNAs or tRNAs; the rest is noncoding DNA, including **pseudogenes** and **repetitive DNA** of unknown function.
- The most abundant type of repetitive DNA in multicellular eukaryotes consists of **transposable elements** and related sequences. In eukaryotes, there are two types of transposable elements: **transposons**, which move via a DNA intermediate, and **retrotransposons**, which are more prevalent and move via an RNA intermediate.
- Other repetitive DNA includes short, noncoding sequences that are tandemly repeated thousands of times (**simple sequence DNA**, which includes **STRs**); these sequences are especially prominent in centromeres and telomeres, where they probably play structural roles in the chromosome.
- Though many eukaryotic genes are present in one copy per haploid chromosome set, others (most, in some species) are members of a gene family, such as the human globin gene families:



? Explain how the function of transposable elements might account for their prevalence in human noncoding DNA.

CONCEPT 20.5

Duplication, rearrangement, and mutation of DNA contribute to genome evolution (pp. 486–491)

- Errors in cell division can lead to extra copies of all or part of entire chromosome sets, which may then diverge if one set accumulates sequence changes. Polyploidy occurs more often among plants than animals and contributes to speciation.
- The chromosomal organization of genomes can be compared among species, providing information about evolutionary relationships. Within a given species, rearrangements of chromosomes are thought to contribute to the emergence of new species.
- The genes encoding the various related but different globin proteins evolved from one common ancestral globin gene, which duplicated and diverged into α -globin and β -globin ancestral genes. Subsequent duplication and random mutation gave rise to the present globin genes, all of which code for oxygen-binding proteins. The copies of some duplicated genes have diverged so much that the functions of their encoded proteins (such as lysozyme and α -lactalbumin) are now substantially different.
- Rearrangement of exons within and between genes during evolution has led to genes containing multiple copies of similar exons and/or several different exons derived from other genes.
- Movement of transposable elements or recombination between copies of the same element can generate new sequence combinations that are beneficial to the organism. These may alter the functions of genes or their patterns of expression and regulation.

? How could chromosomal rearrangements lead to the emergence of new species?

CONCEPT 20.6

Comparing genome sequences provides clues to evolution and development (pp. 491–496)

- Comparisons of genomes from widely divergent and closely related species provide valuable information about ancient and more recent evolutionary history, respectively. Analysis of single nucleotide polymorphisms (SNPs) and copy-number variants (CNVs) among individuals in a species can also shed light on the evolution of that species.
- Evolutionary developmental (**evo-devo**) biologists have shown that homeotic genes and some other genes associated with animal development contain a **homeobox** region whose sequence is highly conserved among diverse species. Related sequences are present in the genes of plants and yeasts.

? What type of information can be obtained by comparing the genomes of closely related species? Of very distantly related species?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–3 can be found in the Study Area in MasteringBiology.

4. **DRAW IT** Below are the amino acid sequences (using the single-letter code; see Figure 5.14) of four short segments of the *FOXP2* protein from six species: chimpanzee (C), orangutan (O), gorilla (G), rhesus macaque (R), mouse (M), and human (H). These segments contain all of the amino acid differences between the *FOXP2* proteins of these species.

1. ATETI...PKSSD...TSSTT...NARRD
2. ATETI...PKSSE...TSSTT...NARRD
3. ATETI...PKSSD...TSSTT...NARRD
4. ATETI...PKSSD...TSSNT...SARRD
5. ATETI...PKSSD...TSSTT...NARRD
6. VTETI...PKSSD...TSSTT...NARRD

Use a highlighter to color any amino acid that varies among the species. (Color that amino acid in all sequences.)

- (a) The C, G, R sequences are identical. Identify which lines correspond to those sequences.
- (b) The H sequence differs from that of the C, G, R species at two amino acids. Underline the two differences in the H sequence.
- (c) The O sequence differs from the C, G, R sequences at one amino acid (having V instead of A) and from the H sequence at three amino acids. Identify the O sequence.
- (d) In the M sequence, circle the amino acid(s) that differ from the C, G, R sequences, and draw a square around those that differ from the H sequence.



PRACTICE
TEST
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- (e) Primates and rodents diverged between 60 and 100 million years ago, and chimpanzees and humans about 6 million years ago. Compare the amino acid differences between the mouse and the C, G, R species with those between the human and the C, G, R species. What can you conclude?
5. **EVOLUTION CONNECTION** The transcription factor *FOXP2* has been found to be important for vocalization in humans. How would a comparison of the sequences of this protein across primates help us understand the evolution of speech?
 6. **SCIENTIFIC INQUIRY** The scientists mapping the SNPs in the human genome noticed that groups of SNPs tended to be inherited together, in blocks known as haplotypes, ranging in length from 5,000 to 200,000 base pairs. There are as few as four or five commonly occurring combinations of SNPs per haplotype. Integrating what you've learned throughout this chapter and this unit, propose an explanation for this observation.
 7. **WRITE ABOUT A THEME: INFORMATION** The continuity of life is based on heritable information in the form of DNA. In a short essay (100–150 words), explain how mutations in protein-coding genes and regulatory DNA contribute to evolution.
 8. **SYNTHESIZE YOUR KNOWLEDGE**



Insects have three thoracic (trunk) segments. While researchers have found insect fossils with pairs of wings on all three segments, modern insects have wings or related structures on only the second and third segment. It turns out that in modern insects, *Hox* gene products act to inhibit wing formation on the first segment. The treehopper insect (above) is somewhat of an exception. In addition to having wings on its second segment, the treehopper's first segment has an ornate helmet that resembles a set of thorns, which a recent study has found to be a modified, fused pair of "wings." The thorn-like structure helps to camouflage the treehopper in tree branches, thus reducing its risk of predation. Explain how changes in gene regulation could have led to the evolution of such a structure.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

UNIT 4

EVOLUTION

A Nobel Prize laureate, Jack W. Szostak is Professor of Genetics at Harvard Medical School and Professor of Chemistry and Chemical Biology at Harvard University and is the Alexander Rich Distinguished Investigator at Massachusetts General Hospital. Dr. Szostak grew up in Canada and received a B.Sc. from McGill University and a Ph.D. in biochemistry from Cornell University. He has made pioneering contributions to genetics, including identifying the role of programmed DNA double-strand breaks in meiotic recombination and the discovery of how chromosomes are protected by telomeres. A member of the National Academy of Sciences, Dr. Szostak is now conducting groundbreaking research on the origin of life on Earth.



An Interview with Jack Szostak

You won a Nobel Prize for your work on telomeres. How did you transition from that topic to studying the origin of life?

After working on telomeres and DNA damage repair in yeast, I started to look around for something new to study. At that time, Sid Altman and Tom Cech had just discovered ribozymes, which are RNA molecules that can function as enzyme-like catalysts. I found this really exciting, and so I switched the focus of my lab to RNA biochemistry. At first, we looked at molecular evolution. But it's one thing to study evolution in the lab, where you, the experimenter, control the conditions under which an existing ribozyme can evolve.

I started to wonder: How in the first place did RNA form and replicate itself on early Earth? I became more and more interested in that question, and now my whole lab is working on it.

What approaches do you use to study the origin of life?

We're using chemical experiments to try to figure out the conditions under which a primitive cell could form, grow, and divide. Much work from other laboratories has contributed to our current understanding of how the chemical building blocks of biology could have been made on early Earth. This has set the stage for exploring the next question: How did these inanimate chemicals assemble into larger structures and start acting like a living cell?

What hurdles remain to answer this question?

The first living cells must have been extremely simple. But they also must have had some of the universal features of cells today, such as a cell membrane and a genetic material like RNA. We've learned how to assemble cell-like structures with a membrane that surrounds RNA. The membranes of these structures can grow and produce "offspring" similar to themselves. The big remaining hurdle is to discover conditions under which RNA can replicate itself in a way that is compatible with a primitive cell membrane.

What can you learn about present-day cells by studying the origin of life?

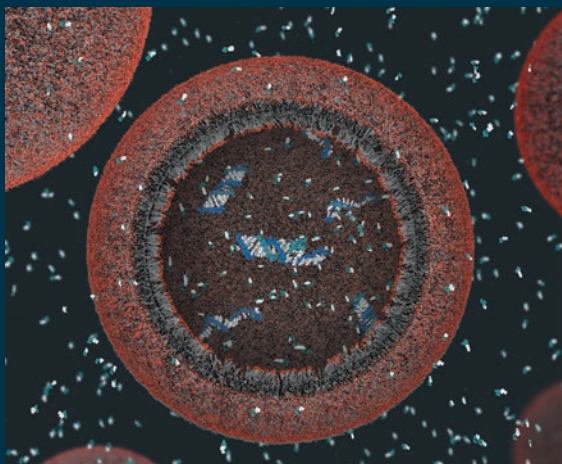
All present-day life shares many features, including the same underlying biochemistry. We're trying to find explanations for such shared features. We've learned that the essential role of RNA seems to emerge naturally from chemical conditions like those on early Earth. But questions remain. For example, all cells maintain certain concentration gradients across their membranes, such as higher potassium levels inside the cell than out, and lower sodium levels inside than out. Cells spend a lot of energy maintaining these gradients—why is that? We don't have a good answer for that yet, but we hope to learn more about such puzzling features of life today by studying its origins.

What do you find most rewarding about your job?

I especially enjoy talking with people about new experiments. The lab is filled with talented students and post-docs who have different backgrounds and different scientific interests. Everyone has to learn how to talk with each other. This makes the lab an exciting and fun place where we all are working together to solve a big scientific problem.

"All present-day life shares many features, including the same underlying biochemistry. We're trying to find explanations for such shared features."

▼ **Model of a protocell containing nucleotides and short bits of RNA**



How Evolution Works

21



▲ **Figure 21.1** How is its resemblance to a fallen leaf helpful to this moth?

KEY CONCEPTS

- 21.1** The Darwinian revolution challenged traditional views of a young Earth inhabited by unchanging species
- 21.2** Descent with modification by natural selection explains the adaptations of organisms and the unity and diversity of life
- 21.3** Evolution is supported by an overwhelming amount of scientific evidence

▼ Juvenile stage (caterpillar) of the dead-leaf moth



Endless Forms Most Beautiful

A hungry bird in the Peruvian rain forest would have to look very closely to spot a “dead-leaf moth” (*Oxytenis modestia*), which blends in well with its forest floor habitat (**Figure 21.1**). This distinctive moth is a member of a diverse group, the more than 120,000 species of lepidopteran insects (moths and butterflies). All lepidopterans have a juvenile stage characterized by a well-developed head and many chewing mouthparts: the ravenous, efficient feeding machines we call caterpillars. (The caterpillar stage of the dead-leaf moth is also protected by its appearance: When threatened, it weaves its head back and forth, resembling a snake about to strike.) As adults, all lepidopterans share other features, such as three pairs of legs and two pairs of wings covered with small scales. But the many lepidopterans also differ from one another. How did there come to be so many different moths and butterflies, and what causes their similarities and differences?

The moth in **Figure 21.1** and its many close relatives illustrate three key observations about life:

- the striking ways in which organisms are suited for life in their environments (Here and throughout this text, the term *environment* refers to other organisms as well as to the physical aspects of an organism’s surroundings.)
- the many shared characteristics (unity) of life
- the rich diversity of life

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

More than a century and a half ago, Charles Darwin was inspired to develop a scientific explanation for these three broad observations. When he published his hypothesis in his book *The Origin of Species*, Darwin ushered in a scientific revolution—the era of evolutionary biology.

For now, we will define **evolution** as *descent with modification*, a phrase Darwin used in proposing that Earth's many species are descendants of ancestral species that were different from the present-day species. Evolution can also be defined as a change in the genetic composition of a population from generation to generation (see Concept 23.3).

We can also view evolution in two related but different ways: as a pattern and as a process. The *pattern* of evolutionary change is revealed by data from many scientific disciplines, including biology, geology, physics, and chemistry. These data are facts—they are observations about the natural world—and these observations show that life has evolved over time. The *process* of evolution consists of the mechanisms that cause the observed pattern of change. These mechanisms represent natural causes of the natural phenomena we observe. Indeed, the power of evolution as a unifying theory is its ability to explain and connect a vast array of observations about the living world.

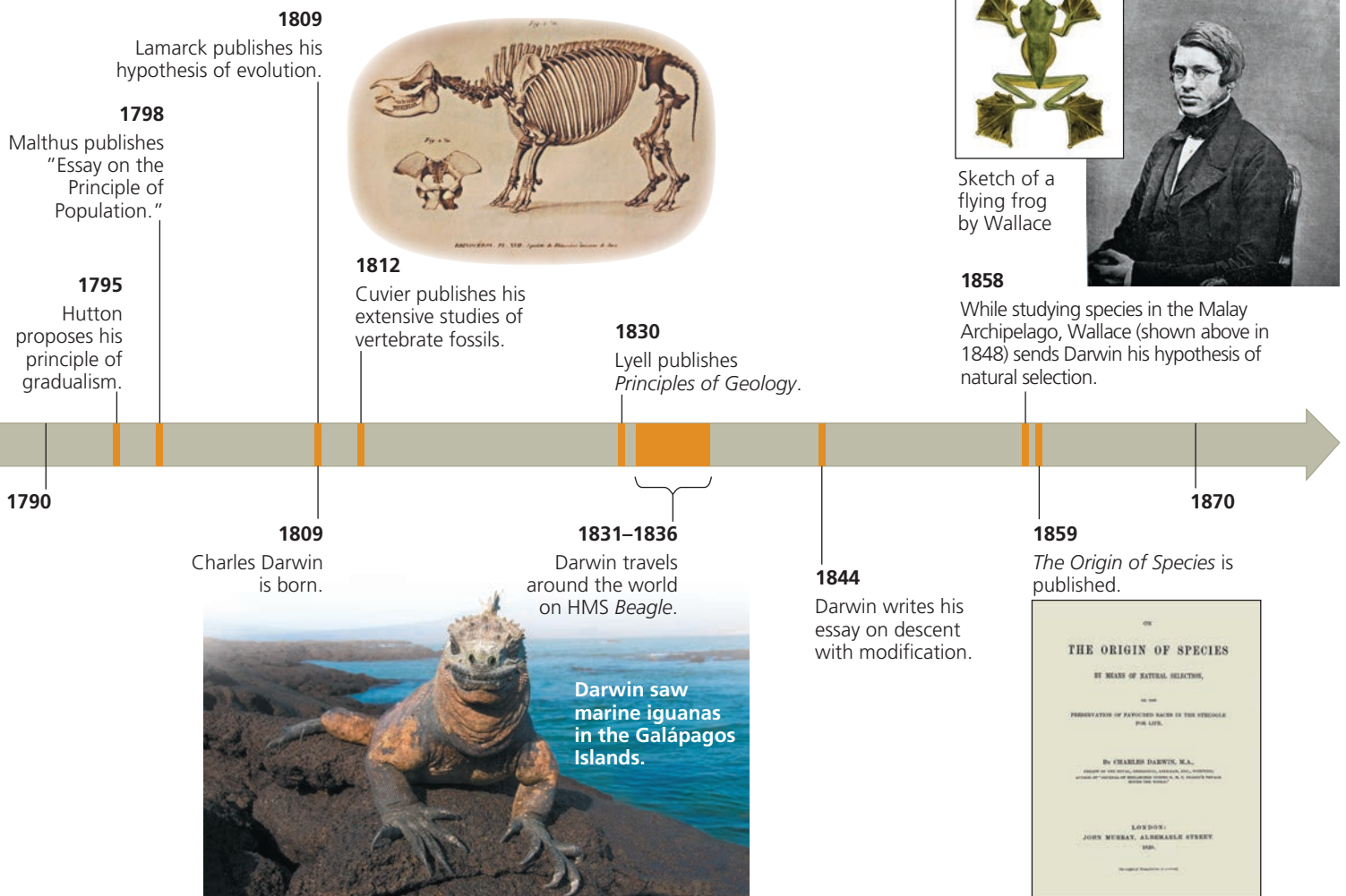
As with all general theories in science, we continue to test our understanding of evolution by examining whether it can account for new observations and experimental results. In this and the following chapters, we'll examine how ongoing discoveries shape what we know about the pattern and process of evolution. To set the stage, we'll first retrace Darwin's quest to explain the adaptations, unity, and diversity of what he called life's "endless forms most beautiful."

CONCEPT 21.1

The Darwinian revolution challenged traditional views of a young Earth inhabited by unchanging species

What impelled Darwin to challenge the prevailing views about Earth and its life? Darwin developed his revolutionary proposal over time, influenced by the work of others and by his travels (Figure 21.2). As we'll see, his ideas also had deep historical roots.

▼ **Figure 21.2** The intellectual context of Darwin's ideas.



Scala Naturae and Classification of Species

Long before Darwin was born, several Greek philosophers suggested that life might have changed gradually over time. But one philosopher who greatly influenced early Western science, Aristotle (384–322 BCE), viewed species as fixed (unchanging). Through his observations of nature, Aristotle recognized certain “affinities” among organisms. He concluded that life-forms could be arranged on a ladder, or scale, of increasing complexity, later called the *scala naturae* (“scale of nature”). Each form of life, perfect and permanent, had its allotted rung on this ladder.

These ideas were generally consistent with the Old Testament account of creation, which holds that species were individually designed by God and therefore perfect. In the 1700s, many scientists interpreted the often remarkable match of organisms to their environment as evidence that the Creator had designed each species for a particular purpose.

One such scientist was Carolus Linnaeus (1707–1778), a Swedish physician and botanist who sought to classify life’s diversity, in his words, “for the greater glory of God.” In the 1750s, Linnaeus developed the two-part, or *binomial*, format for naming species (such as *Homo sapiens* for humans) that is still used today. In contrast to the linear hierarchy of the *scala naturae*, Linnaeus adopted a nested classification system, grouping similar species into increasingly general categories. For example, similar species are grouped in the same genus, similar genera (plural of genus) are grouped in the same family, and so on.

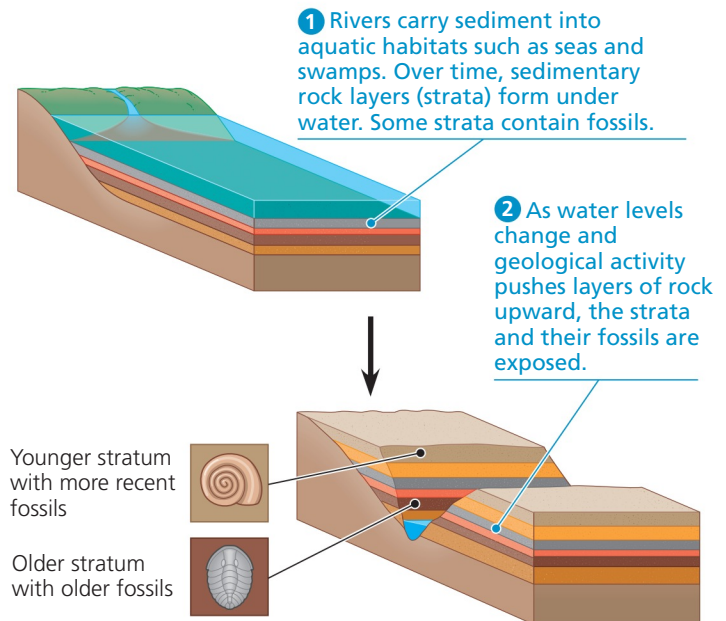
Linnaeus did not ascribe the resemblances among species to evolutionary kinship, but rather to the pattern of their creation. A century later, however, Darwin argued that classification should be based on evolutionary relationships. He also noted that scientists using the Linnaean system often grouped organisms in ways that reflected those relationships.

Ideas About Change over Time

Among other sources of information, Darwin drew from the work of scientists studying **fossils**, the remains or traces of organisms from the past. Many fossils are found in sedimentary rocks formed from the sand and mud that settle to the bottom of seas, lakes, and swamps (Figure 21.3). New layers of sediment cover older ones and compress them into superimposed layers of rock called **strata** (singular, *stratum*). The fossils in a particular stratum provide a glimpse of some of the organisms that populated Earth at the time that layer formed. Later, erosion may carve through upper (younger) strata, revealing deeper (older) strata that had been buried.

Paleontology, the study of fossils, was developed in large part by French scientist Georges Cuvier (1769–1832). In examining strata near Paris, Cuvier noted that the older the stratum, the more dissimilar its fossils were to current life-forms. He also observed that from one layer to the next, some new species

▼ **Figure 21.3** Formation of sedimentary strata with fossils.



 **Video: Grand Canyon**

appeared while others disappeared. He inferred that extinctions must have been a common occurrence, but he staunchly opposed the idea of evolution. Cuvier speculated that each boundary between strata represented a sudden catastrophic event, such as a flood, that had destroyed many of the species living in that area. Such regions, he reasoned, were later repopulated by different species immigrating from other areas.

In contrast to Cuvier’s emphasis on sudden events, other scientists suggested that profound change could take place through the cumulative effect of slow but continuous processes. In 1795, Scottish geologist James Hutton (1726–1797) proposed that Earth’s geologic features could be explained by gradual mechanisms, such as valleys being formed by rivers. The leading geologist of Darwin’s time, Charles Lyell (1797–1875), incorporated Hutton’s thinking into his proposal that the same geologic processes are operating today as in the past, and at the same rate.

Hutton’s and Lyell’s ideas strongly influenced Darwin’s thinking. Darwin agreed that if geologic change results from slow, continuous actions rather than from sudden events, then Earth must be much older than the widely accepted age of a few thousand years. It would, for example, take a very long time for a river to carve a canyon by erosion. He later reasoned that perhaps similarly slow and subtle processes could produce substantial biological change. However, Darwin was not the first to apply the idea of gradual change to biological evolution.

Lamarck’s Hypothesis of Evolution

Although some 18th-century naturalists suggested that life evolves as environments change, only one proposed a mechanism for *how* life changes over time: French biologist

Jean-Baptiste de Lamarck (1744–1829). Alas, Lamarck is primarily remembered today *not* for his visionary recognition that evolutionary change explains patterns in fossils and the match of organisms to their environments, but for the incorrect mechanism he proposed.

Lamarck published his hypothesis in 1809, the year Darwin was born. By comparing living species with fossil forms, Lamarck had found what appeared to be several lines of descent, each a chronological series of older to younger fossils leading to a living species. He explained his findings using two principles that were widely accepted at the time. The first was *use and disuse*, the idea that parts of the body that are used extensively become larger and stronger, while those that are not used deteriorate. Among many examples, he cited a giraffe stretching its neck to reach leaves on high branches. The second principle, *inheritance of acquired characteristics*, stated that an organism could pass these modifications to its offspring. Lamarck reasoned that the long, muscular neck of the living giraffe had evolved over many generations as giraffes stretched their necks ever higher.

Lamarck also thought that evolution happens because organisms have an innate drive to become more complex. Darwin rejected this idea, but he, too, thought that variation was introduced into the evolutionary process in part through inheritance of acquired characteristics. Today, however, our understanding of genetics refutes this mechanism: Experiments show that traits acquired by use during an individual's life are not inherited in the way proposed by Lamarck (**Figure 21.4**).

► **Figure 21.4 Acquired traits cannot be inherited.** This bonsai tree was “trained” to grow as a dwarf by pruning and shaping. However, seeds from this tree would produce offspring of normal size.



Lamarck was vilified in his own time, especially by Cuvier, who denied that species ever evolve. In retrospect, however, Lamarck did recognize that the fact that organisms are well-suited for life in their environments can be explained by gradual evolutionary change, and he did propose a testable explanation for how this change occurs.

CONCEPT CHECK 21.1

1. How did Hutton's and Lyell's ideas influence Darwin's thinking about evolution?
2. **MAKE CONNECTIONS** ► Scientific hypotheses must be testable (see Concept 1.3). Applying this criterion, are Cuvier's explanation of the fossil record and Lamarck's hypothesis of evolution scientific? Explain your answer in each case.

For suggested answers, see Appendix A.

CONCEPT 21.2

Descent with modification by natural selection explains the adaptations of organisms and the unity and diversity of life

As the 19th century dawned, it was generally thought that species had remained unchanged since their creation. A few clouds of doubt about the permanence of species were beginning to gather, but no one could have forecast the thundering storm just beyond the horizon. How did Charles Darwin become the lightning rod for a revolutionary view of life?

Darwin's Research

Charles Darwin (1809–1882) was born in Shrewsbury, in western England. Even as a boy, he had a consuming interest in nature. When he was not reading nature books, he was fishing, hunting, riding, and collecting insects. However, Darwin's father, a physician, could see no future for his son as a naturalist and sent him to medical school in Edinburgh. But Charles found medicine boring and surgery before the days of anesthesia horrifying. He quit medical school and enrolled at Cambridge University, intending to become a clergyman. (At that time, many scholars of science belonged to the clergy.)

At Cambridge, Darwin became the protégé of John Henslow, a botany professor. Soon after Darwin graduated, Henslow recommended him to Captain Robert FitzRoy, who was preparing the survey ship *HMS Beagle* for a long voyage around the world. Darwin would pay his own way and serve as a conversation partner to the young captain. FitzRoy, who was himself an accomplished scientist, accepted Darwin because he was a skilled naturalist and because they were of similar age and social class.

The Voyage of the Beagle

Darwin embarked from England on the *Beagle* in December 1831. The primary mission of the voyage was to chart poorly known stretches of the South American coastline. Darwin, however, spent most of his time on shore, observing and collecting thousands of plants and animals. He described features of organisms that made them well suited to such diverse environments as the humid jungles of Brazil, the expansive grasslands of Argentina, and the towering peaks of the Andes. He also noted that the plants and animals in temperate regions of South America more closely resembled species living in the South American tropics than species living in temperate regions of Europe. Furthermore, the fossils he found, though clearly different from living species, distinctly resembled the living organisms of South America.

Darwin also spent much time thinking about geology. Despite repeated bouts of seasickness, he read Lyell's *Principles of Geology* during the voyage. He experienced geologic change firsthand when a violent earthquake shook the coast of Chile, and he observed afterward that rocks along the coast had been thrust upward by several meters. Finding fossils of ocean organisms high in the Andes, Darwin inferred that the rocks containing the fossils must have been raised there by many similar earthquakes. These observations reinforced what he had learned from Lyell: Physical evidence did not support the traditional view that Earth was only a few thousand years old.

Darwin's interest in the species (or fossils) found in an area was further stimulated by the *Beagle's* stop at the

Galápagos, a group of volcanic islands located near the equator about 900 km west of South America (**Figure 21.5**). Darwin was fascinated by the unusual organisms there. The birds he collected included several kinds of mockingbirds. These mockingbirds, though similar to each other, seemed to be different species. Some were unique to individual islands, while others lived on two or more adjacent islands. Furthermore, although the animals on the Galápagos resembled species living on the South American mainland, most of the Galápagos species were not known from anywhere else in the world. Darwin hypothesized that the Galápagos had been colonized by organisms that had strayed from South America and then diversified, giving rise to new species on the various islands.

Darwin's Focus on Adaptation

During the voyage of the *Beagle*, Darwin observed many examples of **adaptations**, inherited characteristics of organisms that enhance their survival and reproduction in specific environments. Later, as he reassessed his observations, he began to perceive adaptation to the environment and the origin of new species as closely related processes. Could a new species arise from an ancestral form by the gradual accumulation of adaptations to a different environment? From studies made years after Darwin's voyage, biologists have concluded that this is indeed what happened to a diverse group of finches found on the Galápagos Islands (see Figure 1.20). The finches' various beaks and behaviors are adapted to the specific foods available on their home

▼ **Figure 21.5** The voyage of HMS *Beagle* (December 1831–October 1836).

Darwin in 1840, after his return from the voyage



ABC News Video: Protecting the Galápagos Islands

▼ **Figure 21.6 Three examples of beak variation in Galápagos finches.** The Galápagos Islands are home to more than a dozen species of closely related finches, some found only on a single island. A striking difference among them is their beaks, which are adapted for specific diets.



(a) **Cactus-eater.** The long, sharp beak of the common cactus finch (*Geospiza scandens*) helps it tear and eat cactus flowers and pulp.



(b) **Insect-eater.** The green warbler finch (*Certhidea olivacea*) uses its narrow, pointed beak to grasp insects.



(c) **Seed-eater.** The large ground finch (*Geospiza magnirostris*) has a large beak adapted for cracking seeds found on the ground.

MAKE CONNECTIONS ► Review Figure 1.20. Circle the most recent common ancestor shared by the three species that eat insects. Are all of the descendants of that ancestor insect-eaters?

islands (**Figure 21.6**). Darwin realized that explaining such adaptations was essential to understanding evolution. His explanation of how adaptations arise centered on **natural selection**, a process in which individuals that have certain inherited traits tend to survive and reproduce at higher rates than do other individuals *because of* those traits.

By the early 1840s, Darwin had worked out the major features of his hypothesis. He set these ideas on paper in 1844, when he wrote a long essay on descent with modification and its underlying mechanism, natural selection. Yet he was still reluctant to publish his ideas, in part because he anticipated the uproar they would cause. During this time, Darwin continued to compile evidence in support of his hypothesis. By the mid-1850s, he had described his ideas to Lyell and a few others. Lyell, who was not yet convinced of evolution, nevertheless urged Darwin to publish on the subject before someone else came to the same conclusions and published first.

In June 1858, Lyell's prediction came true. Darwin received a manuscript from Alfred Russel Wallace (1823–1913), a British naturalist working in the South Pacific islands of the Malay Archipelago (see Figure 21.2). Wallace had developed a hypothesis of natural selection nearly identical to Darwin's. He asked Darwin to evaluate his paper and forward it to Lyell if it merited publication. Darwin complied, writing to Lyell: "Your words have come true with a vengeance. . . I never saw a more striking coincidence . . . so all my originality, whatever it may amount to, will be smashed." On July 1, 1858, Lyell and a colleague presented Wallace's paper, along with extracts from Darwin's unpublished 1844 essay, to the Linnean Society of London. Darwin quickly finished his book, titled *On the Origin of Species by Means of Natural Selection* (commonly referred to as *The Origin of Species*), and published it the next year. Although Wallace had submitted his ideas for publication first,

he admired Darwin and thought that Darwin had developed and tested the idea of natural selection so extensively that he should be known as its main architect.

Within a decade, Darwin's book and its proponents had convinced most scientists of the time that life's diversity is the product of evolution. Darwin succeeded where previous evolutionists had failed, mainly by presenting a plausible scientific mechanism with immaculate logic and an avalanche of supporting evidence.

 **HHMI Video: The Origin of Species: The Making of a Theory**



Ideas from *The Origin of Species*

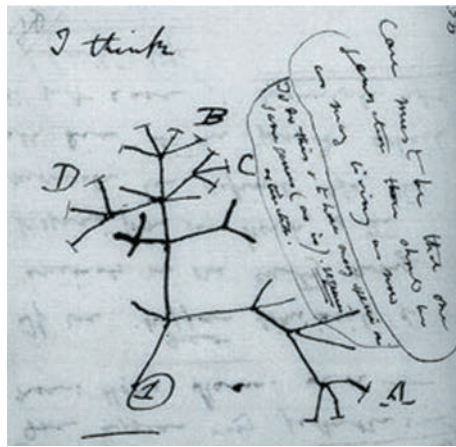
In his book, Darwin amassed evidence that descent with modification by natural selection explains three broad observations about nature—the unity of life, the diversity of life, and the striking ways in which organisms are suited for life in their environments.

Descent with Modification

In the first edition of *The Origin of Species*, Darwin never used the word *evolution* (although the final word of the book is "evolved"). Rather, he discussed *descent with modification*, a phrase that summarized his view of life. Organisms share many characteristics, leading Darwin to perceive unity in life. He attributed the unity of life to the descent of all organisms from an ancestor that lived in the remote past. He also thought that as the descendants of that ancestral organism lived in various habitats, they gradually accumulated diverse modifications, or adaptations, that fit them to specific ways of life. Darwin reasoned that over a long period of time, descent with modification eventually led to the rich diversity of life we see today.

► **Figure 21.7**
“I think . . .”
In this 1837
sketch, Darwin
envisioned
the branching
pattern of
evolution.

Branches that end in twigs labeled A–D represent particular groups of living organisms; all other branches represent extinct groups.



Darwin viewed the history of life as a tree, with multiple branchings from a common trunk out to the tips of the youngest twigs (**Figure 21.7**). In his diagram, the tips of the twigs that are labeled A–D represent several groups of organisms living in the present day, while the unlabeled branches represent groups that are extinct. Each fork of the tree represents the most recent common ancestor of all the lines of evolution that subsequently branch from that point.

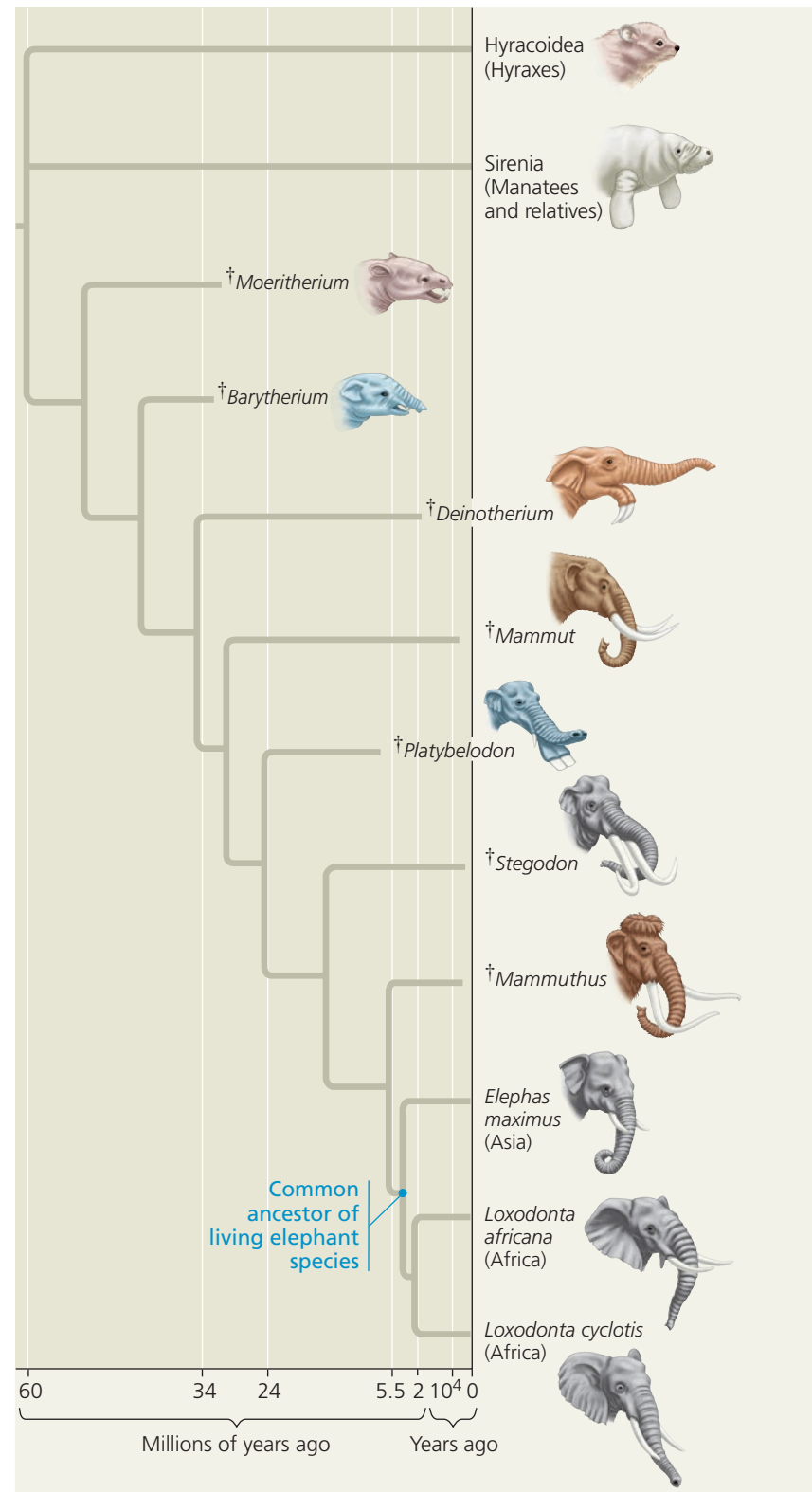
Darwin thought that such a branching process, along with past extinction events, could explain the large morphological gaps that sometimes exist between related groups of organisms. As an example, let’s consider the three living species of elephants: the Asian elephant (*Elephas maximus*) and two species of African elephants (*Loxodonta africana* and *L. cyclotis*). These closely related species are very similar because they shared the same line of descent until a relatively recent split from their common ancestor, as shown in the tree diagram in **Figure 21.8**. Note that seven lineages related to elephants have become extinct over the past 32 million years. As a result, there are no living species that fill the morphological gap between the elephants and their nearest relatives today, the hyraxes and manatees.

Extinctions like those in **Figure 21.8** are not uncommon. In fact, many evolutionary branches, even some major ones, are dead ends: Scientists estimate that over 99% of all species that have ever lived are now extinct. As in **Figure 21.8**, fossils of extinct species can document the divergence of present-day groups by “filling in” gaps between them.

Artificial Selection, Natural Selection, and Adaptation

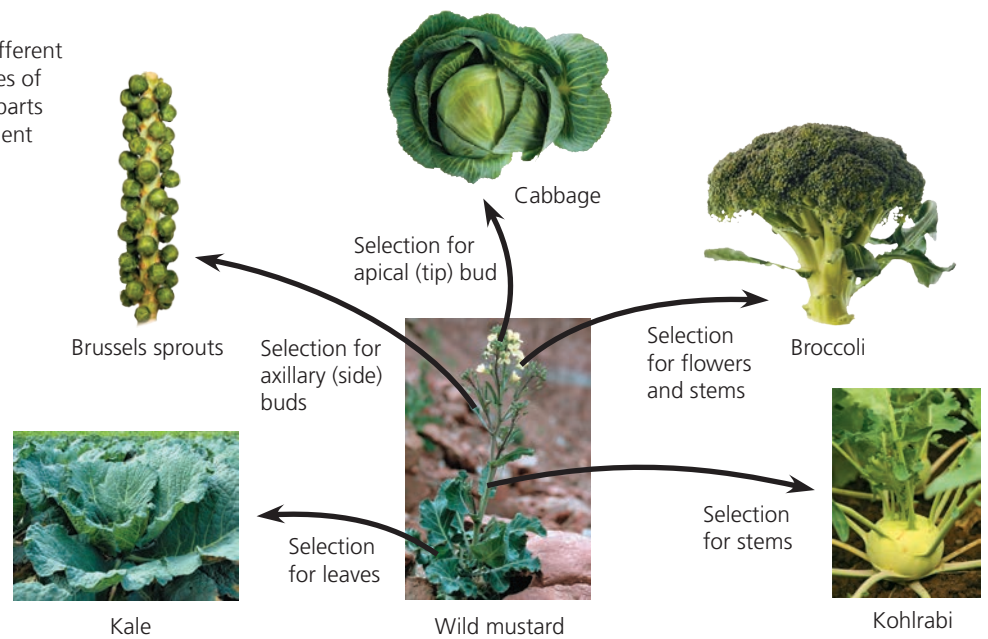
Darwin proposed the mechanism of natural selection to explain the observable patterns of evolution. He crafted his argument carefully, hoping to persuade even the most skeptical readers. First he discussed familiar examples of selective breeding of domesticated plants and animals. Humans have modified other species over many generations by selecting and breeding individuals that possess desired traits, a process

▼ **Figure 21.8** **Descent with modification.** This evolutionary tree of elephants and their relatives is based mainly on fossils—their anatomy, order of appearance in strata, and geographic distribution. Note that most branches of descent ended in extinction (denoted by the dagger symbol, †). (Time line not to scale.)



VISUAL SKILLS ► Based on this tree, approximately when did the most recent ancestor shared by Mammuthus (woolly mammoths), Asian elephants, and African elephants live?

➤ **Figure 21.9 Artificial selection.** These different vegetables have all been selected from one species of wild mustard. By selecting variations in different parts of the plant, breeders have obtained these divergent results.



called **artificial selection** (Figure 21.9). As a result of artificial selection, crops, livestock animals, and pets often bear little resemblance to their wild ancestors.

Darwin then argued that a similar process occurs in nature. He based his argument on two observations, from which he drew two inferences:

Observation #1: Members of a population often vary in their inherited traits (Figure 21.10).

Observation #2: All species can produce more offspring than their environment can support (Figure 21.11), and many of these offspring fail to survive and reproduce.

Inference #1: Individuals whose inherited traits give them a higher probability of surviving and reproducing in a given environment tend to leave more offspring than do other individuals.

Inference #2: This unequal ability of individuals to survive and reproduce will lead to the accumulation of favorable traits in the population over generations.

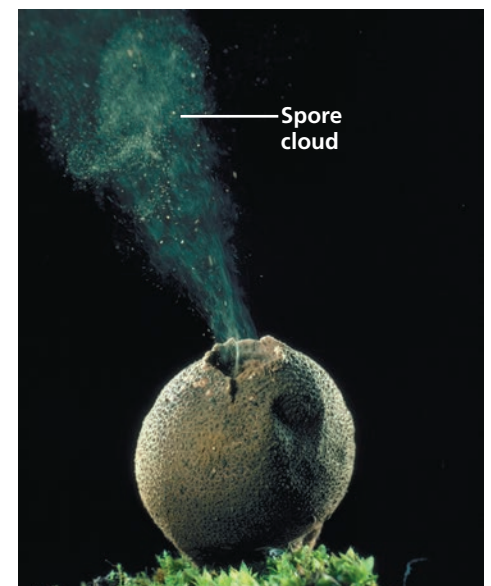
As these two inferences suggest, Darwin saw an important connection between natural selection and the capacity of organisms to “overreproduce.” He began to make this connection after reading an essay by economist Thomas Malthus, who contended that much of human suffering—disease, famine, and war—resulted from the human population’s potential to increase faster than food supplies and other resources. Similarly, Darwin realized that the capacity to overreproduce was characteristic of all species. Of the many eggs laid, young born, and seeds spread, only a tiny fraction complete their development and leave offspring of their own. The rest are eaten, starved, diseased, unmated, or unable to tolerate physical conditions of the environment such as salinity or temperature.

✓ **Figure 21.10 Variation in a population.** Individuals in this population of Asian ladybird beetles vary in color and spot pattern. Natural selection may act on these variations only if (1) they are heritable and (2) they affect the beetles’ ability to survive and reproduce.



➤ **Figure 21.11 Overproduction of offspring.**

A single puffball fungus can produce billions of spores that give rise to offspring. If all of these offspring and their descendants survived to maturity, they would carpet the surrounding land surface.



An organism's heritable traits can influence not only its own performance, but also how well its offspring cope with environmental challenges. For example, an organism might have a trait that gives its offspring an advantage in escaping predators, obtaining food, or tolerating physical conditions. When such advantages increase the number of offspring that survive and reproduce, the traits that are favored will likely appear at a greater frequency in the next generation. Thus, over time, natural selection resulting from factors such as predators, lack of food, or adverse physical conditions can lead to an increase in the proportion of favorable traits in a population.

How rapidly do such changes occur? Darwin reasoned that if artificial selection can bring about dramatic change in a relatively short period of time, then natural selection should be capable of substantial modification of species over many hundreds of generations. Even if the advantages of some heritable traits over others are slight, the advantageous variations will gradually accumulate in the population, and less favorable variations will diminish. Over time, this process will increase the frequency of individuals with favorable adaptations, hence increasing the degree to which organisms are well suited for life in their environment.

Key Features of Natural Selection

Let's now recap the main ideas of natural selection:

- Natural selection is a process in which individuals that have certain heritable traits survive and reproduce at a higher rate than do other individuals because of those traits.
- Over time, natural selection can increase the frequency of adaptations that are favorable in a given environment (**Figure 21.12**).
- If an environment changes, or if individuals move to a new environment, natural selection may result in adaptation to these new conditions, sometimes giving rise to new species.

One subtle but important point is that although natural selection occurs through interactions between individual organisms and their environment, *individuals do not evolve*. Rather, it is the population that evolves over time.

A second key point is that natural selection can amplify or diminish only those heritable traits that differ among the individuals in a population. Thus, even if a trait is heritable, if all the individuals in a population are genetically identical for that trait, evolution by natural selection cannot occur.

Third, remember that environmental factors vary from place to place and over time. A trait that is favorable in one place or time may be useless—or even detrimental—in other places or times. Natural selection is always operating, but which traits are favored depends on the context in which a species lives and mates.

▼ **Figure 21.12 Camouflage as an example of evolutionary adaptation.** Related species of the insects called mantises have diverse shapes and colors that evolved in different environments, as seen in this South African flower-eyed mantis (*Pseudocreobotra wahlbergi*; top) and Malaysian orchid mantis (*Hymenopus coronatus*; bottom).



VISUAL SKILLS ► Use evidence from these two images to explain how these mantises demonstrate the three key observations about life introduced at the beginning of this chapter: the unity and diversity of life and the match between organisms and their environments.

Next, we'll survey the wide range of observations that support a Darwinian view of evolution by natural selection.

CONCEPT CHECK 21.2

1. How does the concept of descent with modification explain both the unity and diversity of life?
2. **WHAT IF?** ► If you discovered a fossil of an extinct mammal that lived high in the Andes, would you predict that it would more closely resemble present-day mammals from South American jungles or present-day mammals that live high in Asian mountains? Explain.
3. **MAKE CONNECTIONS** ► Review the relationship between genotype and phenotype (see Figures 14.5 and 14.6). Suppose that in a particular pea population, flowers with the white phenotype are favored by natural selection. Predict what would happen over time to the frequency of the *p* allele in the population, and explain your reasoning.

For suggested answers, see Appendix A.

CONCEPT 21.3

Evolution is supported by an overwhelming amount of scientific evidence

In *The Origin of Species*, Darwin marshaled a broad range of evidence to support the concept of descent with modification. Still—as he readily acknowledged—there were instances in which key evidence was lacking. For example, Darwin referred to the origin of flowering plants as an “abominable mystery,” and he lamented the lack of fossils showing how earlier groups of organisms gave rise to new groups.

In the last 150 years, new discoveries have filled many of the gaps that Darwin identified. The origin of flowering plants, for example, is much better understood (see Concept 30.3), and many fossils have been discovered that signify the origin of new groups of organisms (see Concept 25.2). In this section, we’ll consider four types of data that document the pattern of evolution and illuminate how it occurs: direct observations, homology, the fossil record, and biogeography.

Direct Observations of Evolutionary Change

Biologists have documented evolutionary change in thousands of scientific studies. We’ll examine many such studies throughout this unit, but let’s look at two examples here.

Natural Selection in Response to Introduced Species

Animals that eat plants, called herbivores, often have adaptations that help them feed efficiently on their primary food sources. What happens when herbivores switch to a new food source with different characteristics?

An opportunity to study this question in nature is provided by soapberry bugs, which use their “beak”—a hollow, needle-like mouthpart—to feed on seeds located within the fruits of various plants. In southern Florida, the soapberry bug (*Jadera haematoloma*) feeds on the seeds of a native plant, the balloon vine (*Cardiospermum corindum*). In central Florida, however, balloon vines have become rare. Instead, soapberry bugs in that region now feed on the seeds of the goldenrain tree (*Koelreuteria elegans*), a species recently introduced from Asia.

Soapberry bugs feed most effectively when the length of their beak is similar to the depth at which seeds are found within the fruit. Goldenrain tree fruit consists of three flat lobes, and its seeds are much closer to the fruit surface than are the seeds of the plump, round fruit of the native balloon vine. These differences led researchers to predict that in populations that feed on goldenrain tree, natural selection would result in beaks that are *shorter* than those in populations that feed on balloon vine (Figure 21.13). Indeed, beak lengths are shorter in the populations that feed on goldenrain tree.

▼ Figure 21.13

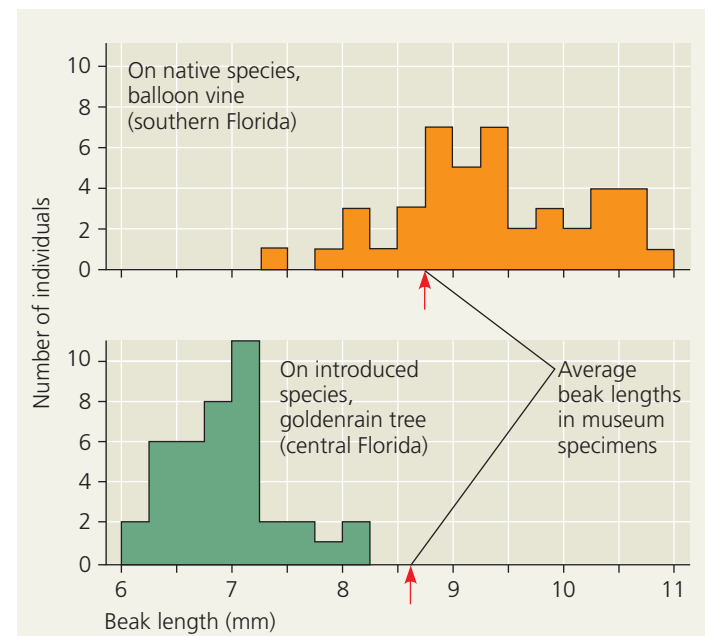
Inquiry Can a change in a population’s food source result in evolution by natural selection?

Field Study Soapberry bugs feed most effectively when the length of their “beak” is similar to the depth of the seeds within the fruit. Scott Carroll and his colleagues measured beak lengths in soapberry bug populations feeding on the native balloon vine. They also measured beak lengths in populations feeding on the introduced goldenrain tree. The researchers then compared the measurements with those of museum specimens collected in the two areas before the goldenrain tree was introduced.



Soapberry bug with beak inserted in balloon vine fruit

Results Beak lengths were shorter in populations feeding on the introduced species than in populations feeding on the native species, in which the seeds are buried more deeply. The average beak length in museum specimens from each population (indicated by red arrows) was similar to beak lengths in populations feeding on native species.



Conclusion Museum specimens and contemporary data suggest that a change in the size of the soapberry bug’s food source can result in evolution by natural selection for a corresponding change in beak size.

Data from S. P. Carroll and C. Boyd, Host race radiation in the soapberry bug: natural history with the history, *Evolution* 46:1052–1069 (1992).

WHAT IF? Data from additional studies showed that when soapberry bug eggs from a population that fed on balloon vine fruits were then reared on goldenrain tree fruits (or vice versa), the beak lengths of the adult insects were most similar to those in the population from which the eggs were initially obtained. Interpret these results.

Researchers have also studied beak length evolution in soapberry bug populations that feed on plants introduced to Louisiana, Oklahoma, and Australia. In each of these locations, the fruit of the introduced plants is larger than the fruit of the native plant. Thus, in populations feeding on introduced species in these regions, researchers predicted that natural selection would result in the evolution of *longer* beaks. Again, data collected in field studies upheld this prediction.

The observed changes in beak lengths had important consequences: In Australia, for example, the increase in beak length nearly doubled the success with which soapberry bugs could eat the seeds of the introduced species. Furthermore, since historical data show that the goldenrain tree reached central Florida just 35 years before the scientific studies were initiated, the results demonstrate that natural selection can cause rapid evolution in a wild population.

The Evolution of Drug-Resistant Bacteria

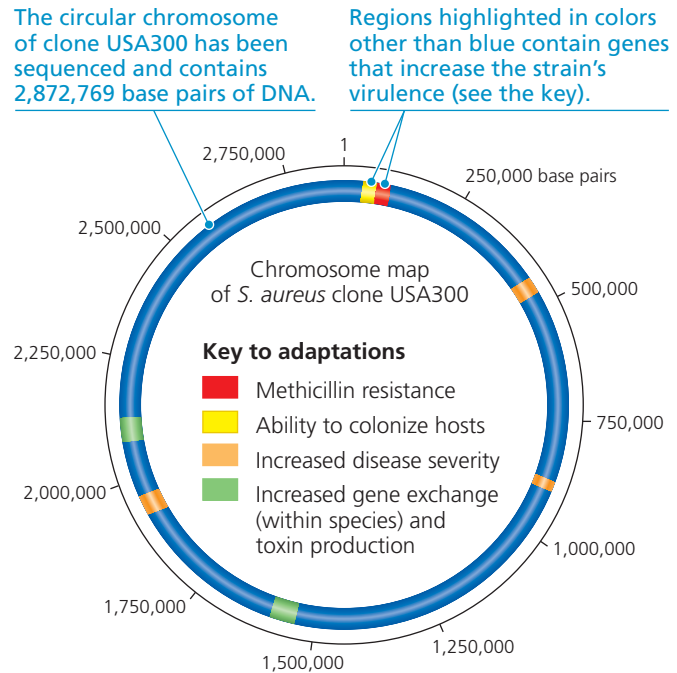
An example of ongoing natural selection that dramatically affects humans is the evolution of drug-resistant pathogens (disease-causing organisms and viruses). This is a particular problem with bacteria and viruses because they can produce new generations in a short period of time; as a result, resistant strains of these pathogens can proliferate very quickly.

Consider the evolution of drug resistance in the bacterium *Staphylococcus aureus*. About one in three people harbor this species on their skin or in their nasal passages with no negative effects. However, certain genetic varieties (strains) of this species, known as methicillin-resistant *S. aureus* (MRSA), are formidable pathogens. Most MRSA infections are caused by recently appearing strains such as clone USA300, which can cause “flesh-eating disease” and potentially fatal infections (**Figure 21.14**). How did clone USA300 and other strains of MRSA become so dangerous?

The story begins in 1943, when penicillin became the first widely used antibiotic. Since then, penicillin and other antibiotics have saved millions of lives. However, by 1945, more than 20% of the *S. aureus* strains seen in hospitals were already resistant to penicillin. These bacteria had an enzyme, penicillinase, that could destroy penicillin. Researchers responded by developing antibiotics that were not destroyed by penicillinase, but resistance to each new drug was observed in some *S. aureus* populations within a few years.

Then, in 1959, doctors began using a promising new antibiotic, methicillin. But within two years, methicillin-resistant strains of *S. aureus* were observed. How did these resistant strains emerge? Methicillin works by deactivating an enzyme that bacteria use to synthesize their cell walls. However, some *S. aureus* populations included individuals that were able to synthesize their cell walls using a different enzyme that was not affected by methicillin. These individuals survived the methicillin treatments and reproduced at higher rates than did other individuals. Over time, these resistant individuals became increasingly common, leading to the spread of MRSA.

▼ Figure 21.14 Clone USA300: a virulent strain of methicillin-resistant *Staphylococcus aureus* (MRSA). Resistant to multiple antibiotics and highly contagious, this strain and its close relatives can cause lethal infections of the skin, lungs, and blood. As shown here, researchers have identified key areas of the USA300 genome that code for adaptations that cause its virulent properties.



WHAT IF? ► Some drugs being developed specifically target and kill only *S. aureus*; others slow the growth of MRSA but do not kill it. Based on how natural selection works and on the fact that bacterial species can exchange genes, explain why each of these strategies might be effective.

Initially, MRSA could be controlled by antibiotics that work differently from the way methicillin works. But this has become less effective because some MRSA strains are resistant to multiple antibiotics—probably because bacteria can exchange genes with members of their own and other species. Thus, the multidrug-resistant strains of today may have emerged over time as MRSA strains that were resistant to different antibiotics exchanged genes.

Finally, it is important to note that *S. aureus* is not the only pathogenic bacterium that has evolved resistance to multiple antibiotics. Furthermore, in recent decades, antibiotic resistance has spread much faster than new antibiotics have been discovered—a problem of great public health concern. Hope may loom on the horizon, however. For example, in 2015, scientists reported the discovery of “teixobactin,” a new antibiotic that shows promise for treating MRSA and other pathogens. In addition, as we’ll describe in the Scientific Skills Exercise in Chapter 27, the methods used in the discovery of teixobactin may lead to the discovery of other new antibiotics as well.

The *S. aureus* and soapberry bug examples highlight three key points about natural selection. First, natural selection is a process of editing, not a creative mechanism. A drug does not *create* resistant pathogens; it *selects for* resistant individuals that are already present in the population. Second, in species that produce new generations in short periods of time, evolution

by natural selection can occur rapidly—in just a few years (*S. aureus*) or decades (soapberry bugs). Third, natural selection depends on time and place. It favors those characteristics in a genetically variable population that provide an advantage in the current, local environment. What is beneficial in one situation may be useless or even harmful in another. Beak lengths suitable for the size of the typical fruit eaten by members of a particular soapberry bug population are favored by natural selection. However, a beak length suitable for fruit of one size can be disadvantageous when the bug is feeding on fruit of another size.

Homology

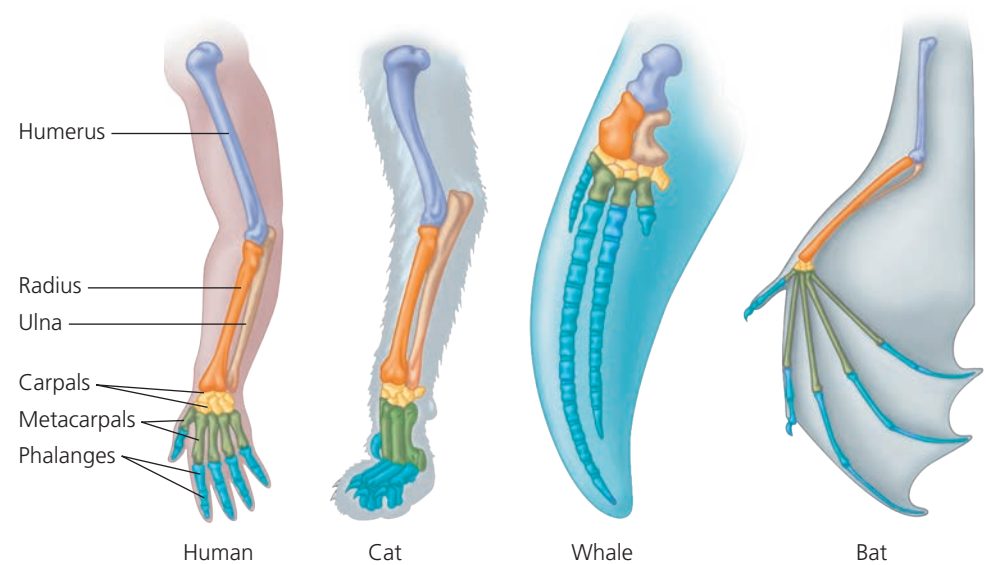
A second type of evidence for evolution comes from analyzing similarities among different organisms. As we've discussed, evolution is a process of descent with modification: Characteristics present in an ancestral organism are altered (by natural selection) in its descendants over time as they face different environmental conditions. As a result, related species can have characteristics that have an underlying similarity yet function differently. Similarity resulting from common ancestry is known as **homology**. As we'll describe in this section, an understanding of homology can be used to make testable predictions and explain observations that are otherwise puzzling.

Anatomical and Molecular Homologies

The view of evolution as a remodeling process leads to the prediction that closely related species should share similar features—and they do. Of course, closely related species share the features used to determine their relationship, but they also share many other features. Some of these shared features make little sense except in the context of evolution. For example, the forelimbs of all mammals, including humans, cats, whales, and bats, show the same arrangement of bones from the shoulder to the tips of the digits, even though the appendages have very different functions: lifting, walking, swimming, and flying (**Figure 21.15**). Such striking anatomical resemblances would be highly unlikely if these structures had arisen anew in each species. Rather, the underlying skeletons of the arms, forelegs, flippers, and wings of different mammals are **homologous structures** that represent variations on a structural theme that was present in their common ancestor.

Comparing early stages of development in different animal species reveals additional anatomical homologies not

✓ **Figure 21.15 Mammalian forelimbs: homologous structures.** Even though they have become adapted for different functions, the forelimbs of all mammals are constructed from the same basic skeletal elements: one large bone (purple), attached to two smaller bones (orange and tan), attached to several small bones (gold), attached to several metacarpals (green), attached to approximately five digits, each of which is composed of multiple phalanges (blue).

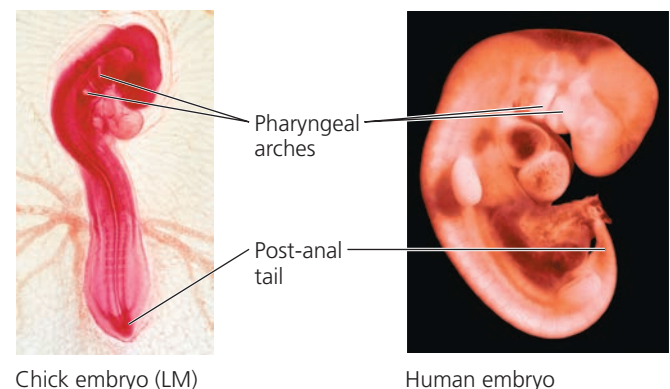


visible in adult organisms. For example, at some point in their development, all vertebrate embryos have a tail located posterior to (behind) the anus, as well as structures called pharyngeal (throat) arches (**Figure 21.16**). These homologous throat arches ultimately develop into structures with very different functions, such as gills in fishes and parts of the ears and throat in humans and other mammals.

Some of the most intriguing homologies concern “leftover” structures of marginal, if any, importance to the organism. These **vestigial structures** are remnants of features that served a function in the organism's ancestors. For instance, the skeletons of some snakes retain vestiges of the pelvis and leg bones of walking ancestors. Another example is provided by eye remnants that are buried under scales in blind species

✓ **Figure 21.16 Anatomical similarities in vertebrate embryos.**

At some stage in their embryonic development, all vertebrates have a tail located posterior to the anus (referred to as a post-anal tail), as well as pharyngeal (throat) arches. Descent from a common ancestor can explain such similarities.



of cave fishes. We would not expect to see these vestigial structures if snakes and blind cave fishes had origins separate from those of other vertebrate animals.

Biologists also observe similarities among organisms at the molecular level. All forms of life use essentially the same genetic code, suggesting that all species descended from common ancestors that used this code. But molecular homologies go beyond a shared code. For example, organisms as dissimilar as humans and bacteria share genes inherited from a very distant common ancestor. Some of these homologous genes have acquired new functions, while others, such as those coding for the ribosomal subunits used in protein synthesis (see Figure 17.18), have retained their original functions. It is also common for organisms to have genes that have lost their function, even though the homologous genes in related species may be fully functional. Like vestigial structures, it appears that such inactive “pseudogenes” may be present simply because a common ancestor had them.

Homologies and “Tree Thinking”

Some homologous characteristics, such as the genetic code, are shared by all species because they date to the deep ancestral past. In contrast, homologous characteristics that evolved more recently are shared only within smaller groups of organisms. Consider the *tetrapods* (from the Greek *tetra*, four, and *pod*, foot), the vertebrate group that consists of amphibians, mammals, and reptiles. Like all vertebrates, tetrapods have a backbone. But unlike other vertebrates, tetrapods also have limbs with digits (see Figure 21.15). As suggested by this example, homologous characteristics form a nested pattern: All life shares the deepest layer (in this case, all vertebrates have a backbone), and each successive smaller group adds its own homologies to those it shares with larger groups (in this case, all tetrapods have a backbone *and* limbs with digits). This nested pattern is exactly

what we would expect to result from descent with modification from a common ancestor.

Biologists often represent the pattern of descent from common ancestors with an **evolutionary tree**, a diagram that reflects evolutionary relationships among groups of organisms. We will explore evolutionary trees in more detail in Chapter 22, but for now, let’s consider how we can interpret and use such trees.

Figure 21.17 is an evolutionary tree of tetrapods and their closest living relatives, the lungfishes. In this diagram, each branch point represents the most recent common ancestor of the two lineages diverging from that point. For example, lungfishes and all tetrapods descended from ancestor **1**, whereas mammals, lizards and snakes, crocodiles, and birds all descended from ancestor **3**. As expected, the three homologies shown on the tree—limbs with digits, the amnion (a protective embryonic membrane), and feathers—form a nested pattern. Limbs with digits were present in common ancestor **2** and hence are found in all of the descendants of that ancestor (the tetrapods). The amnion was present only in ancestor **3** and hence is shared only by some tetrapods (mammals and reptiles). Feathers were present only in ancestor **6** and hence are found only in birds.

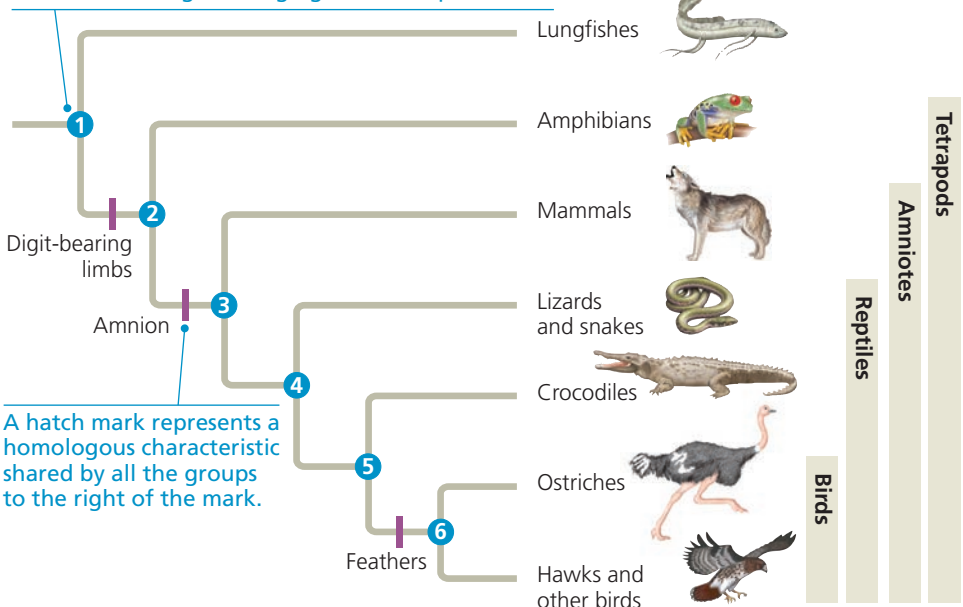
To explore “tree thinking” further, note that in Figure 21.17, mammals are positioned closer to amphibians than to birds. As a result, you might conclude that mammals are more closely related to amphibians than they are to birds. However, mammals are actually more closely related to birds than to amphibians because mammals and birds share a more recent common ancestor (ancestor **3**) than do mammals and amphibians (ancestor **2**). Ancestor **2** is also the most recent common ancestor of birds and amphibians, making mammals and birds equally related to amphibians. Finally, note that the tree in Figure 21.17 shows the relative timing of events but not their actual dates. Thus, we can conclude that ancestor **2** lived before ancestor **3**, but we do not know when that was.

> Figure 21.17 Tree thinking: information provided in an evolutionary tree. This evolutionary tree for tetrapods and their closest living relatives, the lungfishes, is based on anatomical and DNA sequence data. The purple bars indicate the origin of three important homologies, each of which evolved only once. Birds are nested within and evolved from reptiles; hence, the group of organisms called “reptiles” technically includes birds.

VISUAL SKILLS > Based on this evolutionary tree, are crocodiles more closely related to lizards or birds? Explain.



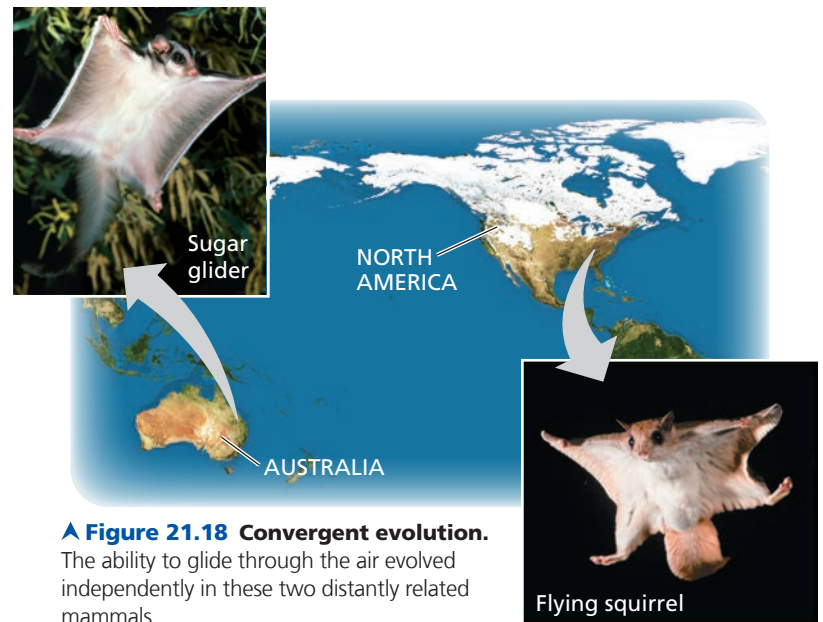
Each branch point represents the common ancestor of the two lineages diverging from that point.



Evolutionary trees are hypotheses that summarize our current understanding of patterns of descent. Our confidence in these relationships, as with any hypothesis, depends on the strength of the supporting data. In the case of Figure 21.17, the tree is supported by many different data sets, including both anatomical and DNA sequence data. As a result, biologists are confident that it accurately reflects evolutionary history. Scientists can use such well-supported evolutionary trees to make specific and sometimes surprising predictions about organisms (see Figure 22.17).

A Different Cause of Resemblance: Convergent Evolution

Although organisms that are closely related share characteristics because of common descent, distantly related organisms can resemble one another for a different reason: **convergent evolution**, the independent evolution of similar features in different lineages. Consider marsupial mammals, many of which live in Australia. Marsupials are distinct from another group of mammals—the placental mammals, or eutherians—few of which live in Australia. (Eutherians complete their embryonic development in the uterus, whereas marsupials are born as embryos and complete their development in an external pouch.) Some Australian marsupials have eutherian look-alikes with superficially similar adaptations. For instance, a forest-dwelling Australian marsupial called the sugar glider is superficially very similar to flying squirrels, gliding eutherians that live in North American forests (Figure 21.18). But the sugar glider has many other characteristics that make it a marsupial, much more closely related to kangaroos and other Australian marsupials than to flying squirrels or other eutherians. Once again, our understanding of evolution can explain these observations. Although they evolved independently from different ancestors, these two mammals have adapted to similar environments in similar ways. In such examples in which species share features because of convergent evolution, the resemblance is said to be **analogous**, not homologous. Analogous features share similar function, but not common ancestry, while homologous features share common ancestry, but not necessarily similar function.



▲ Figure 21.18 Convergent evolution. The ability to glide through the air evolved independently in these two distantly related mammals.

found that over several thousand years, the pelvic bone in fossil stickleback fish became greatly reduced in size. The consistent nature of this change over time suggests that the reduction in the size of the pelvic bone may have been driven by natural selection.

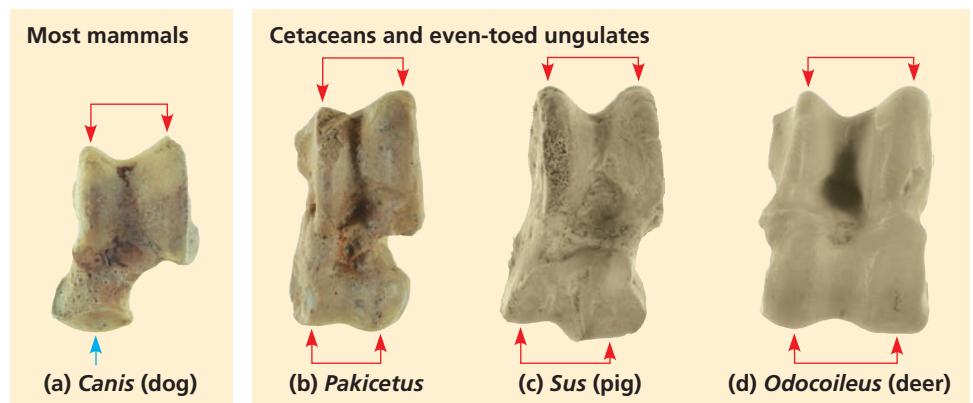
Fossils can also shed light on the origins of new groups of organisms. An example is the fossil record of cetaceans, the mammalian order that includes whales, dolphins, and porpoises. Some of these fossils (Figure 21.19) provided strong support for a hypothesis based on DNA sequence data: that cetaceans are closely related to even-toed ungulates, a group that includes hippopotamuses, pigs, deer, and cows.

What else can fossils tell us about cetacean origins? The earliest cetaceans lived 50–60 million years ago. The fossil record indicates that prior to that time, most mammals were terrestrial. Although scientists had long realized that whales and other cetaceans originated from land mammals, few fossils

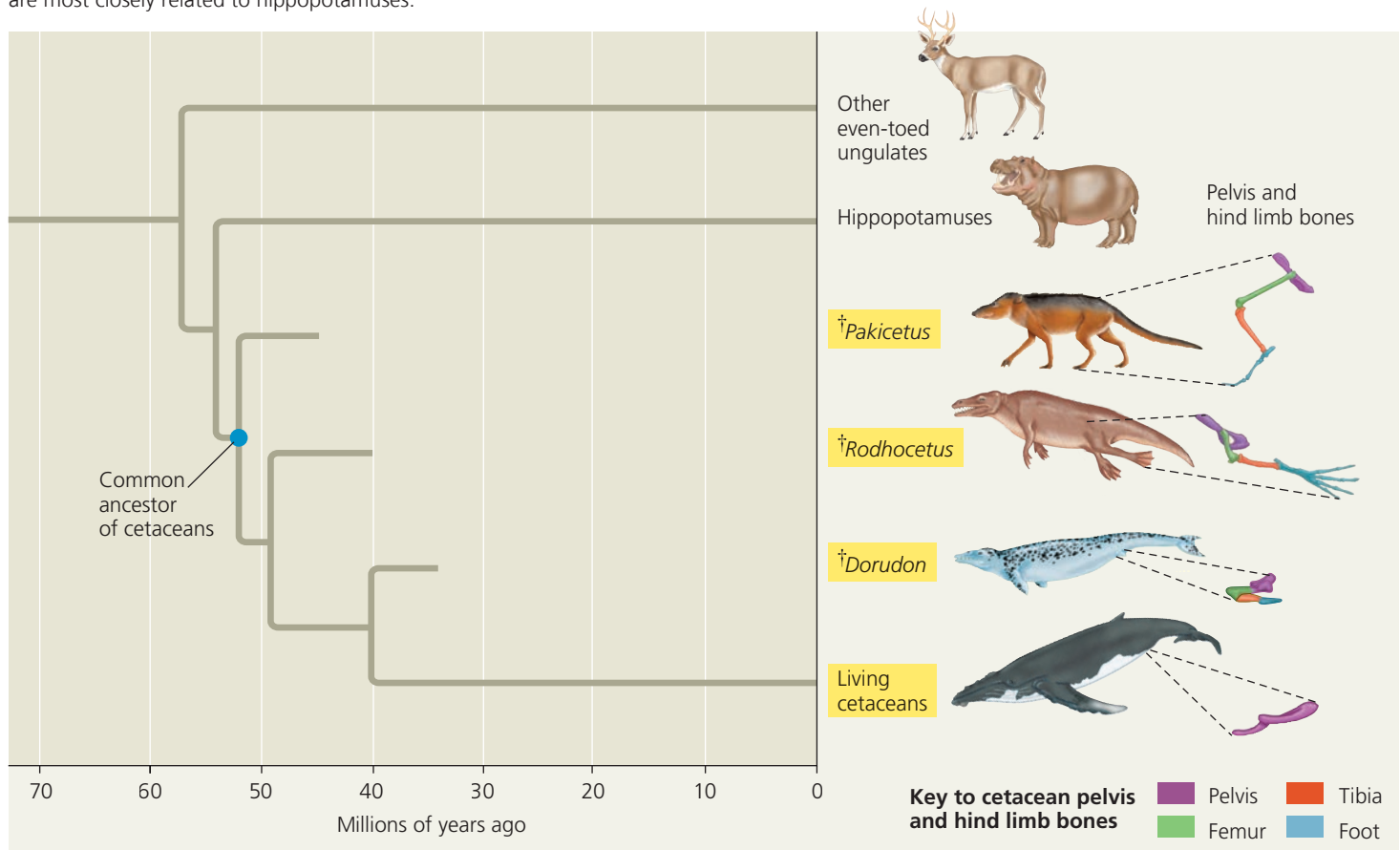
▼ Figure 21.19 Ankle bones: one piece of the puzzle. Comparing fossils and present-day examples of the astragalus (a type of ankle bone) indicates that cetaceans are closely related to even-toed ungulates. **(a)** In most mammals, the astragalus is shaped like that of a dog, with a double hump on one end (red arrows) but not at the opposite end (blue arrow). **(b)** Fossils show that the early cetacean *Pakicetus* had an astragalus with double humps at both ends, a shape otherwise found only in pigs **(c)**, deer **(d)**, and all other even-toed ungulates.

The Fossil Record

A third type of evidence for evolution comes from fossils. The fossil record documents the pattern of evolution, showing that past organisms differed from present-day organisms and that many species have become extinct. Fossils also show the evolutionary changes that have occurred in various groups of organisms. To give one of hundreds of possible examples, researchers



▼ **Figure 21.20 The transition to life in the sea.** Multiple lines of evidence support the hypothesis that cetaceans (highlighted in yellow) evolved from terrestrial mammals. Fossils document the reduction over time in the pelvis and hind limb bones of extinct (†) cetacean ancestors, including *Pakicetus*, *Rodhocetus*, and *Dorudon*. DNA sequence data support the hypothesis that cetaceans are most closely related to hippopotamuses.



VISUAL SKILLS ► Use the diagram to determine which happened first during the evolution of cetaceans: changes in hind limb structure or the origin of tail flukes. Explain.

had been found that revealed how cetacean limb structure had changed over time, leading eventually to the loss of hind limbs and the development of flukes (the lobes on a whale's tail) and flippers. In the past few decades, however, a series of remarkable fossils have been discovered in Pakistan, Egypt, and North America. These fossils document steps in the transition from life on land to life in the sea, filling in some of the gaps between ancestral and living cetaceans (**Figure 21.20**).

Collectively, the recent fossil discoveries document the origin of a group of mammals, the cetaceans. These discoveries also show that cetaceans and their close living relatives (hippopotamuses and other even-toed ungulates) are much more different from each other than were *Pakicetus* and early even-toed ungulates, such as *Diacodexis* (**Figure 21.21**). Similar patterns are seen in fossils documenting the origins of other groups of organisms, including mammals (see Figure 25.7), flowering plants (see Concept 30.3), and tetrapods (see Figure 34.21).

▼ **Figure 21.21** *Diacodexis*, an early even-toed ungulate.



In each of these cases, the fossil record shows that over time, descent with modification produced increasingly large differences among related groups of organisms, ultimately resulting in the diversity of life we see today.

Biogeography

A fourth type of evidence for evolution comes from the field of **biogeography**, the scientific study of the geographic distributions of species. The geographic distributions of organisms are influenced by many factors, including *continental drift*, the slow movement of Earth's continents over time. About 250 million years ago, these movements united all of Earth's landmasses into a single large continent called **Pangaea** (see Figure 25.16). Roughly 200 million years ago, Pangaea began to break apart; by 20 million years ago, the continents we know today were within a few hundred kilometers of their present locations.

We can use our understanding of evolution and continental drift to predict where fossils of different groups of organisms might be found. For example, scientists have constructed evolutionary trees for horses based on anatomical data. These trees and the ages of fossils of horse ancestors

What, then, is theoretical about evolution? Keep in mind that the scientific meaning of the term *theory* is very different from its meaning in everyday use. The colloquial use of the word *theory* comes close to what scientists mean by a hypothesis. In science, a theory is more comprehensive than a hypothesis. A theory, such as the theory of evolution by natural selection, accounts for many observations and explains and integrates a great variety of phenomena. Such a unifying theory does not become widely accepted unless its predictions stand up to thorough and continual testing by experiment and additional observation (see Concept 1.3). As the rest of this unit demonstrates, this has certainly been the case with the theory of evolution by natural selection.

The skepticism of scientists as they continue to test theories prevents these ideas from becoming dogma. For example, although Darwin thought that evolution was a very slow process, we now know that this isn't always true. Populations can evolve rapidly, and new species can form in relatively short periods of time: a few thousand years or less. Furthermore, evolutionary biologists now recognize that natural selection is not the only mechanism responsible for evolution. Indeed, the study of evolution today is livelier than ever as scientists

use a wide range of experimental approaches and genetic analyses to test predictions based on natural selection and other evolutionary mechanisms.

Although Darwin's theory attributes life's diversity to natural processes, the diverse products of evolution are nevertheless elegant and inspiring. As Darwin wrote in the final sentence of *The Origin of Species*, "There is grandeur in this view of life . . . [in which] endless forms most beautiful and most wonderful have been, and are being, evolved."

CONCEPT CHECK 21.3

1. Explain how the following statement is inaccurate: "Antibiotics have created drug resistance in MRSA."
2. How does evolution account for (a) the similar mammalian forelimbs with different functions shown in Figure 21.15 and (b) the similar forms of the two distantly related mammals shown in Figure 21.18?
3. **WHAT IF? >** Fossils show that dinosaurs originated 200–250 million years ago. Would you expect the geographic distribution of early dinosaur fossils to be broad (on many continents) or narrow (on one or a few continents only)? Explain.

For suggested answers, see Appendix A.

21 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 21.1

The Darwinian revolution challenged traditional views of a young Earth inhabited by unchanging species (pp. 501–503)



- Darwin proposed that life's diversity arose from ancestral species through natural selection, a departure from prevailing views.
- Cuvier studied **fossils** but denied that evolution occurs; he proposed that sudden catastrophic events in the past caused species to disappear from an area.
- Hutton and Lyell thought that geologic change could result from gradual mechanisms that operated in the past in the same manner as they do today.
- Lamarck hypothesized that species evolve, but the underlying mechanisms he proposed are not supported by evidence.

? Why was the age of Earth important for Darwin's ideas about evolution?

CONCEPT 21.2

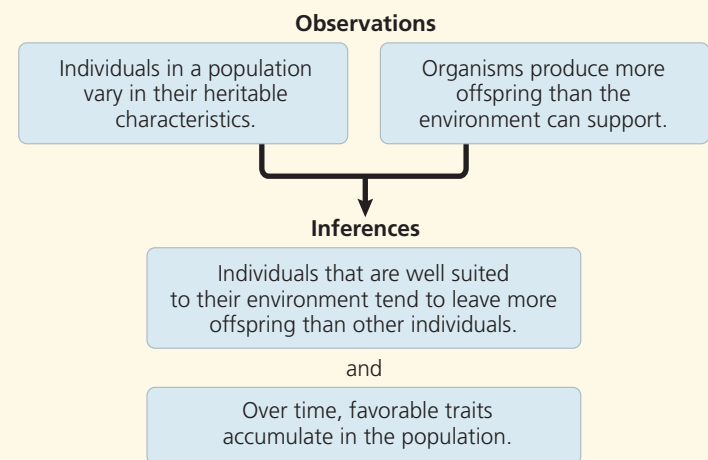
Descent with modification by natural selection explains the adaptations of organisms and the unity and diversity of life (pp. 503–508)

- Darwin's experiences during the voyage of the *Beagle* gave rise to his idea that new species originate from ancestral forms through the accumulation of **adaptations**. He refined his theory for many years and finally published it in 1859 after learning that Wallace had come to the same idea.



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- In *The Origin of Species*, Darwin proposed that over long periods of time, descent with modification produced the rich diversity of life through the mechanism of **natural selection**.



? Describe how overreproduction and heritable variation relate to evolution by natural selection.

CONCEPT 21.3

Evolution is supported by an overwhelming amount of scientific evidence (pp. 509–516)

- Researchers have directly observed natural selection leading to adaptive evolution in many studies, including research on soapberry bug populations and on MRSA.

- Organisms share characteristics because of common descent (**homology**) or because natural selection affects independently evolving species in similar environments in similar ways (**convergent evolution**).
- Fossils show that past organisms differed from living organisms, that many species have become extinct, and that species have evolved over long periods of time; fossils also document the evolutionary origin of new groups of organisms.
- Evolutionary theory can explain some biogeographic patterns.

? Summarize the different lines of evidence supporting the hypothesis that cetaceans descended from land mammals and are closely related to even-toed ungulates.

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

- EVOLUTION CONNECTION** Explain why anatomical and molecular features often fit a similar nested pattern. In addition, describe a process that can cause this not to be the case.
- SCIENTIFIC INQUIRY • DRAW IT** Mosquitoes resistant to the pesticide DDT first appeared in India in 1959, but now are found throughout the world. (a) Graph the data in the table below. (b) Examine the graph, then hypothesize why the percentage of mosquitoes resistant to DDT rose rapidly. (c) Suggest an explanation for the global spread of DDT resistance.



PRACTICE TEST
goo.gl/iAsVgI

Month	0	8	12
Mosquitoes Resistant* to DDT	4%	45%	77%

*Mosquitoes were considered resistant if they were not killed within 1 hour of receiving a dose of 4% DDT.

Data from C. F. Curtis et al., Selection for and against insecticide resistance and possible methods of inhibiting the evolution of resistance in mosquitoes, *Ecological Entomology* 3:273–287 (1978).

- WRITE ABOUT A THEME: INTERACTIONS** Write a short essay (about 100–150 words) evaluating whether changes to an organism's physical environment are likely to result in evolutionary change. Use an example to support your reasoning.

9. SYNTHESIZE YOUR KNOWLEDGE



This honey pot ant (genus *Myrmecocystus*) can store liquid food inside its expandable abdomen. Consider other ants you are familiar with, and explain how a honey pot ant exemplifies three key features of life: adaptation, unity, and diversity.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

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Phylogenetic Reconstruction

22



▲ **Figure 22.1** What kind of organism is this?

KEY CONCEPTS

- 22.1** Phylogenies show evolutionary relationships
- 22.2** Phylogenies are inferred from morphological and molecular data
- 22.3** Shared characters are used to construct phylogenetic trees
- 22.4** An organism's evolutionary history is documented in its genome
- 22.5** Molecular clocks help track evolutionary time
- 22.6** Our understanding of the tree of life continues to change based on new data

Investigating the Tree of Life

Look closely at the organism in **Figure 22.1**. Although it resembles a snake, this animal is actually a legless lizard known as the European glass lizard (*Ophisaurus apodus*). Why isn't this glass lizard considered a snake? More generally, how do biologists distinguish and categorize the millions of species on Earth?

An understanding of evolutionary relationships suggests one way to address these questions: We can decide in which category to place a species by comparing its traits with those of potential close relatives. For example, the glass lizard does not have a highly mobile jaw, a large number of vertebrae, or a short tail located behind the anus, three traits shared by all snakes. These and other characteristics suggest that despite a superficial resemblance, the glass lizard is not a snake.

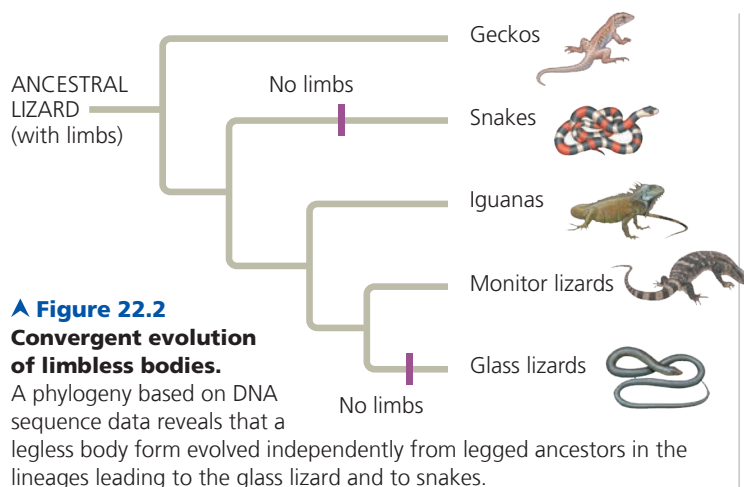
Snakes and lizards are part of the continuum of life extending from the earliest organisms to the great variety of species alive today. In this unit, we will survey this diversity and describe hypotheses regarding how it evolved. As we do so, our emphasis will shift from the *process* of evolution (the evolutionary mechanisms described in Unit Four) to its *pattern* (observations of evolution's products over time).

To set the stage for surveying life's diversity, in this chapter we consider how biologists trace **phylogeny**, the evolutionary history of a species or group of species. A phylogeny of lizards and snakes, for example, indicates that both the eastern glass lizard and snakes evolved from lizards with legs—but they evolved from different

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Get Ready for This Chapter



lineages of legged lizards (Figure 22.2). Thus, it appears that their legless conditions evolved independently. As we'll see, biologists reconstruct phylogenies like that in Figure 22.2 using **systematics**, a discipline focused on classifying organisms and determining their evolutionary relationships.

CONCEPT 22.1

Phylogenies show evolutionary relationships

Organisms share many characteristics because of common ancestry (see Concept 21.3). As a result, we can learn a great deal about a species if we know its evolutionary history. For example, an organism is likely to share many of its genes, metabolic pathways, and structural proteins with its close relatives. We'll consider practical applications of such information later in this section, but first we'll examine how organisms are named and classified, the scientific discipline of **taxonomy**. We'll also look at how we can interpret and use diagrams that represent evolutionary history.

Binomial Nomenclature

Common names for organisms—such as monkey, finch, and lilac—convey meaning in casual usage, but they can also cause confusion. Each of these names, for example, refers to more than one species. Moreover, some common names do not accurately reflect the kind of organism they signify. Consider these three “fishes”: jellyfish (a cnidarian), crayfish (a small, lobsterlike crustacean), and silverfish (an insect). And, of course, a given organism has different names in different languages.

To avoid ambiguity when communicating about their research, biologists refer to organisms by Latin scientific names. The two-part format of the scientific name, commonly called a **binomial**, was instituted in the 18th century by Carolus Linnaeus (see Concept 21.1). The first part of a

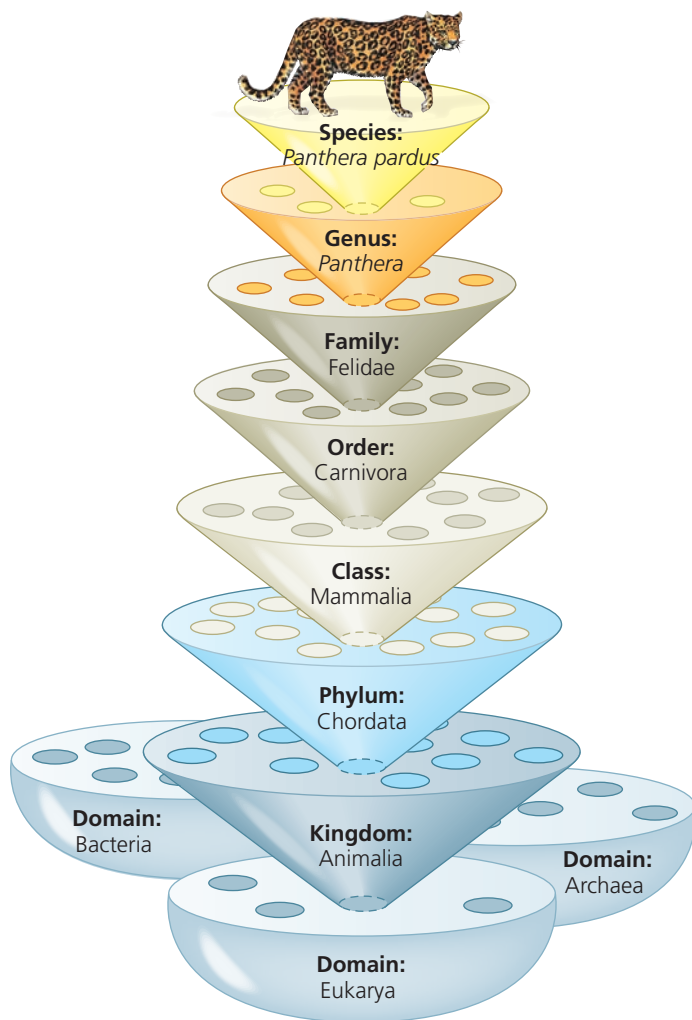
binomial is the name of the **genus** (plural, *genera*) to which the species belongs. The second part, called the specific epithet, is unique for each species within the genus. An example of a binomial is *Panthera pardus*, the scientific name for the leopard. Notice that the first letter of the genus is capitalized and the entire binomial is italicized. (Newly created scientific names are also “latinized”: You can name an insect you discover after a friend, but you must add a Latin ending.) Many of the more than 11,000 binomials assigned by Linnaeus are still used today, including the optimistic name he gave our own species—*Homo sapiens*, meaning “wise man.”

Hierarchical Classification

In addition to naming species, Linnaeus also grouped them into a hierarchy of increasingly inclusive categories. The first grouping is built into the binomial: Species that appear to be closely related are grouped into the same genus. For example, the leopard (*Panthera pardus*) belongs to a genus that also includes the African lion (*Panthera leo*), the tiger (*Panthera tigris*), and the jaguar (*Panthera onca*). Beyond genera, taxonomists employ progressively more comprehensive categories of classification. The taxonomic system named after Linnaeus, the Linnaean system, places related genera in the same **family**, families into **orders**, orders into **classes**, classes into **phyla** (singular, *phylum*), phyla into **kingdoms**, and, more recently, kingdoms into **domains** (Figure 22.3). The resulting biological classification of a particular organism is somewhat like a postal address identifying a person in a particular apartment, in a building with many apartments, on a street with many apartment buildings, in a city with many streets, and so on.

The named taxonomic unit at any level of the hierarchy is called a **taxon** (plural, *taxa*). In the leopard example, *Panthera* is a taxon at the genus level, and Mammalia is a taxon at the class level that includes all the many orders of mammals. Note that in the Linnaean system, taxa broader than the genus are not italicized, though they are capitalized.

Classifying species is a way to structure our human view of the world. We lump together various species of trees to which we give the common name of pines and distinguish them from other trees that we call firs. Taxonomists have decided that pines and firs are different enough to be placed in separate genera, yet similar enough to be grouped into the same family, Pinaceae. As with pines and firs, higher levels of classification are usually defined by particular characters chosen by taxonomists. However, characters that are useful for classifying one group of organisms may not be appropriate for other organisms. For this reason, the larger categories often are not comparable between lineages; that is, an order of snails does not exhibit the same degree of morphological or genetic diversity as an order of mammals. As we'll see, the placement of species into orders, classes, and so on also does not necessarily reflect evolutionary history.



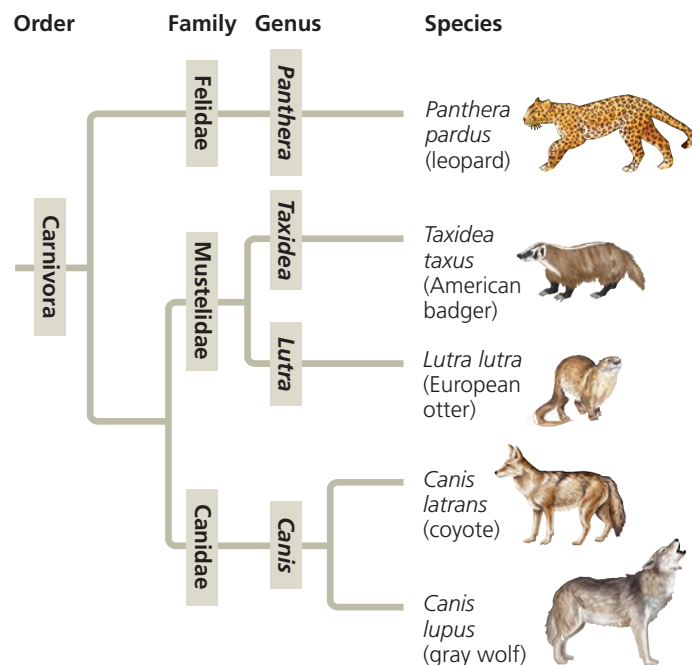
▲ **Figure 22.3 Linnaean classification.** At each level, or “rank,” species are placed in groups within more inclusive groups.

Linking Classification and Phylogeny

The evolutionary history of a group of organisms can be represented in a branching diagram called a **phylogenetic tree**. As in **Figure 22.4**, the branching pattern often matches how taxonomists have classified groups of organisms nested within more inclusive groups. Sometimes, however, taxonomists have placed a species within a genus (or other group) to which it is *not* most closely related. One reason for such a mistake might be that over the course of evolution, a species has lost a key feature shared by its close relatives. If DNA or other new evidence indicates that an organism has been misclassified, the organism may be reclassified to accurately reflect its evolutionary history. Another issue is that while the Linnaean system may distinguish groups, such as amphibians, mammals, reptiles, and other classes of vertebrates, it tells us nothing about these groups’ evolutionary relationships to one another.

Such difficulties in aligning Linnaean classification with phylogeny have led some systematists to propose that

▼ **Figure 22.4 The connection between classification and phylogeny.** Hierarchical classification can reflect the branching patterns of phylogenetic trees. This tree shows evolutionary relationships between some of the taxa within order Carnivora, itself a branch of class Mammalia.



classification be based entirely on evolutionary relationships. In such systems, names are only assigned to groups that include a common ancestor and all of its descendants. As a consequence of this approach, some commonly recognized groups would become part of other groups previously at the same level of the Linnaean system. For example, because birds evolved from a group of reptiles, Aves (the Linnaean class to which birds are assigned) would be considered a subgroup of Reptilia (also a class in the Linnaean system).

What We Can and Cannot Learn from Phylogenetic Trees

Regardless of how groups are named, a phylogenetic tree represents a hypothesis about evolutionary relationships (**Figure 22.5**). These relationships often are depicted as a series of dichotomies, or two-way branch points. Each **branch point** represents the common ancestor of the two evolutionary lineages diverging from it.

In **Figure 22.5**, each tree has a branch point that represents the common ancestor of the lineages leading to chimpanzees and humans. Chimps and humans are considered **sister taxa**, groups of organisms that share an immediate common ancestor that is not shared by any other group. The members of a sister group are each other’s closest relatives, making sister groups a useful way to describe the evolutionary relationships shown in a tree. For example, in **Figure 22.5**, the evolutionary lineage leading to lizards shares an immediate common ancestor with the lineage

▼ Figure 22.5 Visualizing Phylogenetic Relationships

A phylogenetic tree visually represents a hypothesis of how a group of organisms are related. This figure explores how the way a tree is drawn conveys information.



Instructors: Additional questions related to this Visualizing Figure can be assigned in MasteringBiology.

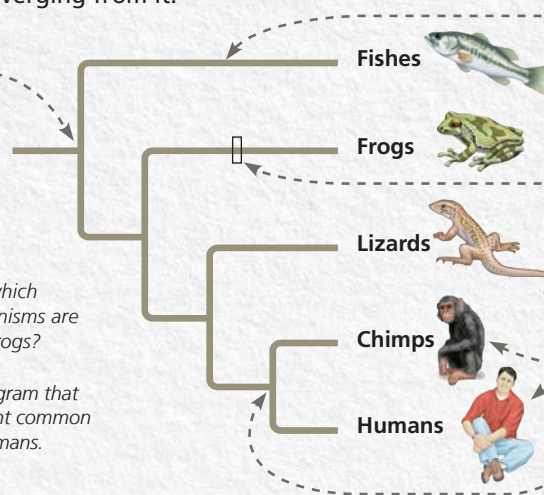
Parts of a Tree

This tree shows how the five groups of organisms at the tips of the branches, called **taxa**, are related. Each **branch point** represents the common ancestor of the evolutionary lineages diverging from it.

This branch point represents the common ancestor of all the animal groups shown in this tree.

1 According to this tree, which group or groups of organisms are most closely related to frogs?

2 Label the part of the diagram that represents the most recent common ancestor of frogs and humans.



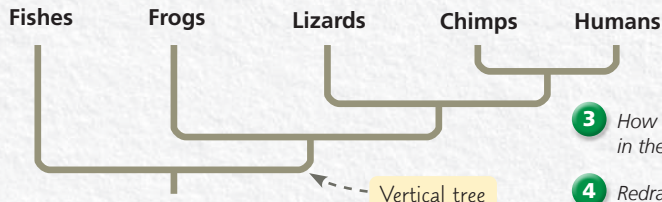
Each horizontal branch represents an **evolutionary lineage**. The length of the branch is arbitrary unless the diagram specifies that branch lengths represent information such as time or amount of genetic change (see Figure 22.13).

Each position along a branch represents an ancestor in the lineage leading to the taxon named at the tip.

Sister taxa are groups of organisms that share a common ancestor that is not shared by any other group. Chimps and humans are an example of sister taxa in this tree.

Alternative Forms of Tree Diagrams

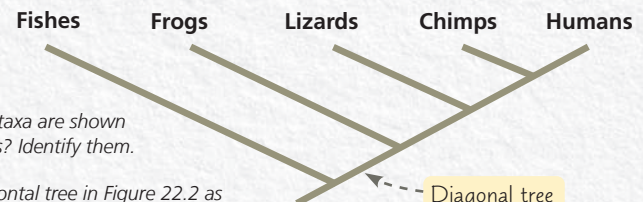
These diagrams are referred to as “trees” because they use the visual analogy of branches to represent evolutionary lineages diverging over time. In this text, trees are usually drawn horizontally, as shown above, but the same tree can be drawn vertically or diagonally without changing the relationships it conveys.



Vertical tree

3 How many sister taxa are shown in these two trees? Identify them.

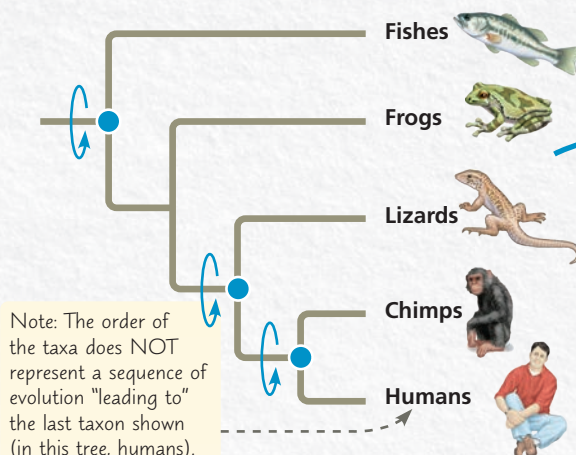
4 Redraw the horizontal tree in Figure 22.2 as a vertical tree and a diagonal tree.



Diagonal tree

Rotating Around Branch Points

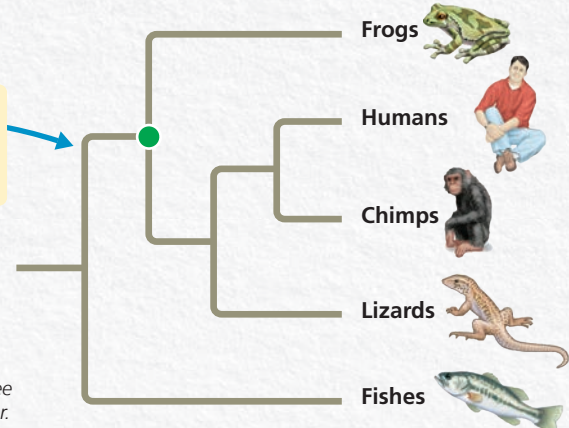
Rotating the branches of a tree around a branch point does not change what they convey about evolutionary relationships. As a result, the order in which taxa appear at the branch tips is not significant. What matters is the branching pattern, which signifies the order in which the lineages have diverged from common ancestors.



Note: The order of the taxa does NOT represent a sequence of evolution “leading to” the last taxon shown (in this tree, humans).

Rotating the branches in the tree at left around the three blue branch points yields the tree on the right.

5 Redraw the tree on the right, rotating around the green branch point. Identify the two closest relatives of humans as shown in each of the three trees. Explain your answer.



leading to chimpanzees and humans. Thus, we can describe this portion of the tree by saying that of the groups shown here, lizards are the sister taxon to a group consisting of chimpanzees and humans.

As also shown in Figure 22.5, the branches of a tree can be rotated around branch points without changing the relationships shown in the tree. That is, the order in which the taxa appear at the right side of the tree does not represent a *sequence* of evolution—in this case, it does not imply a sequence leading from fishes to humans.

This tree, like all of the phylogenetic trees in this book, is **rooted**, which means that a branch point within the tree (often drawn farthest to the left) represents the most recent common ancestor of all taxa in the tree. A lineage that diverges from all other members of its group early in the history of the group is called a **basal taxon**. Hence, like the fishes in Figure 22.5, a basal taxon lies on a branch that diverges near the common ancestor of the group.

What other key points do we need to keep in mind when interpreting phylogenetic trees? First, they are intended to show patterns of descent, not phenotypic similarity. Although closely related organisms often resemble one another due to their common ancestry, they may not if their lineages have evolved at different rates or faced very different environmental conditions. For example, even though crocodiles are more closely related to birds than to lizards (see Figure 21.17), they look more like lizards because morphology has changed dramatically in the bird lineage.

Second, we cannot necessarily infer the ages of the taxa or branch points shown in a tree. For example, the tree in Figure 22.5 does not indicate that chimpanzees evolved before humans. Rather, the tree shows only that chimpanzees and humans share a recent common ancestor, but we cannot tell when that ancestor lived or when the first chimpanzees or humans arose. Generally, unless given specific information about what the branch lengths in a tree mean—for example, that they are proportional to time—we should interpret the diagram solely in terms of patterns of descent. No assumptions should be made about when particular species evolved or how much change occurred in each lineage.

Third, we should not assume that a taxon on a phylogenetic tree evolved from the taxon next to it. Figure 22.5 does not indicate that humans evolved from chimpanzees or vice versa. We can infer only that the lineage leading to humans and the lineage leading to chimpanzees both evolved from a recent common ancestor. That ancestor, which is now extinct, was neither a human nor a chimpanzee.

Applying Phylogenies

Understanding phylogeny can have practical applications. Consider maize (corn), which originated in the Americas and is now an important food crop worldwide. From a phylogeny of maize based on DNA data, researchers have

been able to identify two species of wild grasses that may be maize's closest living relatives. These two close relatives may be useful as “reservoirs” of beneficial alleles that can be transferred to cultivated maize by cross-breeding or genetic engineering.

A different use of phylogenetic trees is to infer species identities by analyzing the relatedness of DNA sequences from different organisms. Researchers have used this approach to investigate whether “whale meat” had been harvested illegally from whale species protected under international law rather than from species that can be harvested legally (Figure 22.6).

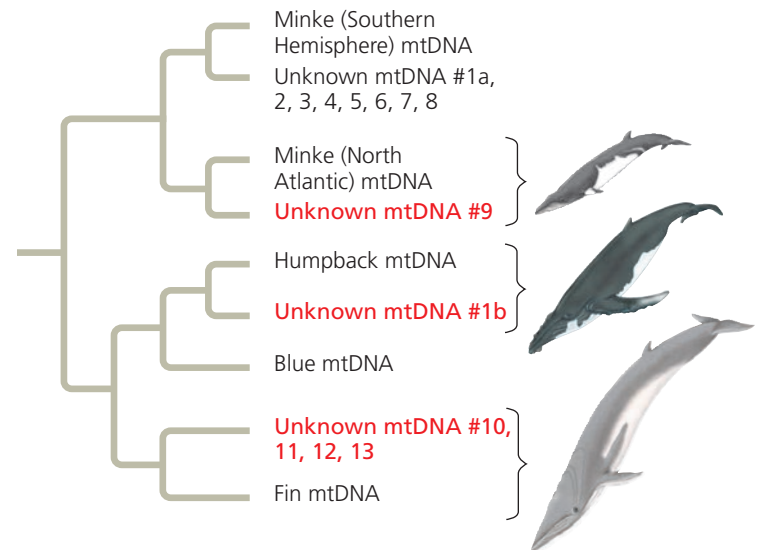
How do researchers construct phylogenetic trees like those we've considered here? In the next section, we'll begin to answer that question by examining the data used to determine phylogenies.

▼ Figure 22.6

Inquiry What is the species identity of food being sold as whale meat?

Experiment C. S. Baker and S. R. Palumbi purchased 13 samples of “whale meat” from Japanese fish markets. They sequenced part of the mitochondrial DNA (mtDNA) from each sample and compared their results with the comparable mtDNA sequence from known whale species. To infer the species identity of each sample, the team constructed a *gene tree*, a phylogenetic tree that shows patterns of relatedness among DNA sequences rather than among taxa.

Results Of the species in the resulting gene tree, only Minke whales caught in the Southern Hemisphere can be sold legally in Japan.

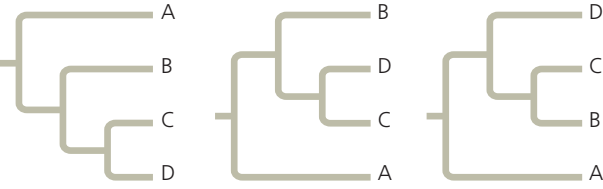


Conclusion This analysis indicated that mtDNA sequences of six of the unknown samples (in red) were most closely related to mtDNA sequences of whales that are not legal to harvest.

Data from C. S. Baker and S. R. Palumbi, Which whales are hunted? A molecular genetic approach to monitoring whaling, *Science* 265:1538–1539 (1994). Reprinted with permission from AAAS.

WHAT IF? > What different results would have indicated that all of the whale meat had been harvested legally?

CONCEPT CHECK 22.1

- VISUAL SKILLS** > Which levels of the classification in Figure 22.3 do humans share with leopards?
- VISUAL SKILLS** > Which of the trees shown here depicts an evolutionary history different from the other two? Explain.

- DRAW IT** > The bear family (Ursidae) is more closely related to the badger/otter family (Mustelidae) than to the dog family (Canidae). Use this information to redraw Figure 22.4.

For suggested answers, see Appendix A.

CONCEPT 22.2

Phylogenies are inferred from morphological and molecular data

To infer phylogeny, systematists must gather as much information as possible about the morphology, genes, and biochemistry of the relevant organisms. It is important to focus on features that result from common ancestry because only those features reflect evolutionary relationships.

Morphological and Molecular Homologies

Recall that phenotypic and genetic similarities due to shared ancestry are called **homologies**. For example, the similarity in the number and arrangement of bones in the forelimbs of mammals is due to their descent from a common ancestor with the same bone structure; this is an example of a morphological homology (see Figure 21.15). In the same way, genes or other DNA sequences are homologous if they are descended from sequences carried by a common ancestor.

In general, organisms that share very similar morphologies or similar DNA sequences are likely to be more closely related than organisms with vastly different structures or sequences. In some cases, however, the morphological divergence between related species can be great and their genetic divergence small (or vice versa). Consider the Hawaiian silversword plants: some of these species are tall, twiggy trees, while others are dense, ground-hugging shrubs (see Figure 25.22). But despite these striking phenotypic differences, the silverswords' genes are very similar. Based on these small molecular divergences, scientists estimate that the silversword group began to diverge 5 million years ago. We'll discuss how scientists use molecular data to estimate such divergence times later in this chapter.

Sorting Homology from Analogy

A potential source of confusion in constructing a phylogeny is similarity between organisms that is due to convergent evolution—called **analogy**—rather than to shared ancestry (homology). Convergent evolution occurs when similar environmental pressures and natural selection produce similar (analogous) adaptations in organisms from different evolutionary lineages.

For example, the two mole-like animals shown in **Figure 22.7** look very similar. However, their internal anatomy, physiology, and reproductive systems are very dissimilar. Indeed, genetic and fossil evidence indicate that the common ancestor of these animals lived 140 million years ago. This common ancestor and most of its descendants were not mole-like. It appears that analogous characteristics evolved independently in these two lineages as they became adapted to similar lifestyles—hence, the similar features of these animals should not be considered when reconstructing their phylogeny.

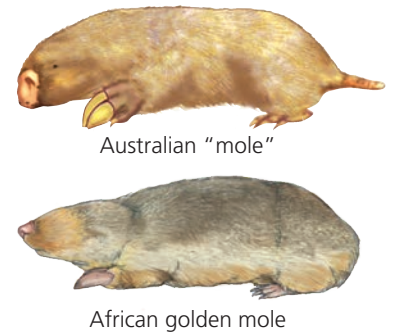
Another clue to distinguishing between homology and analogy is the complexity of the characters being compared. The more elements that are similar in two complex structures, the more likely it is that the structures evolved from a common ancestor. For instance, the skulls of an adult human and an adult chimpanzee both consist of many bones fused together. The compositions of the skulls match almost perfectly, bone for bone. It is highly improbable that such complex structures, matching in so many details, have separate origins. More likely, the genes involved in the development of both skulls were inherited from a common ancestor.

The same argument applies to comparisons at the gene level. Genes are sequences of thousands of nucleotides, each of which represents an inherited character in the form of one of the four DNA bases: A (adenine), G (guanine), C (cytosine), or T (thymine). If genes in two organisms share many portions of their nucleotide sequences, it is likely that the genes are homologous.

Evaluating Molecular Homologies

Comparing DNA molecules often poses technical challenges for researchers. The first step after sequencing the molecules is to align comparable sequences from the species being studied. If the species are very closely related, the sequences probably differ at only one or a few sites. In contrast, comparable nucleic acid sequences in distantly related species

Figure 22.7 Convergent evolution in burrowers. A long body, large front paws, small eyes, and a pad of thick skin that protects the nose all evolved independently in these species.



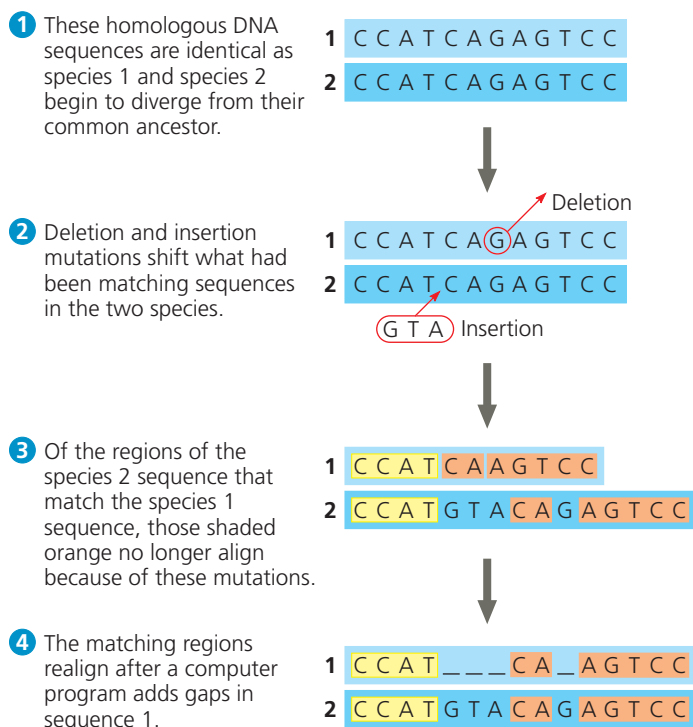
usually have different bases at many sites and may have different lengths. This is because insertions and deletions accumulate over long periods of time.

Suppose, for example, that certain noncoding DNA sequences near a particular gene are very similar in two species, except that the first base of the sequence has been deleted in one of the species. The effect is that the remaining sequence shifts back one notch. A comparison of the two sequences that does not take this deletion into account would overlook what in fact is a very good match. To address such problems, researchers have developed computer programs that estimate the best way to align comparable DNA segments of differing lengths (**Figure 22.8**).

Such molecular comparisons reveal that many base substitutions and other differences have accumulated in the comparable genes of an Australian “mole” and a golden mole. The many differences indicate that their lineages have diverged greatly since their common ancestor; thus, we say that the living species are not closely related. In contrast, the high degree of gene sequence similarity among the silversword plants indicates that they are all very closely related, in spite of their considerable morphological differences.

Just as with morphological characters, it is necessary to distinguish homology from analogy in evaluating molecular similarities for evolutionary studies. Two sequences that resemble each other at many points along their length most

▼ **Figure 22.8 Aligning segments of DNA.** Systematists search for similar sequences along DNA segments from two species (only one DNA strand is shown for each species). In this example, 11 of the original 12 bases have not changed since the species diverged. Hence, those portions of the sequences still align once the length is adjusted.



▼ **Figure 22.9 A molecular homoplasy.**

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A C G G A T A G T C C A C T A G G C A C T A
T C A C C G A C A G G T C T T T G A C T A G

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likely are homologous (see Figure 22.8). But in organisms that do not appear to be closely related, the bases that their otherwise very different sequences happen to share may simply be coincidental matches, called molecular homoplasies. For example, if the two DNA sequences in **Figure 22.9** were from distantly related organisms, the fact that they share 23% of their bases would be coincidental. Statistical tools have been developed to determine whether DNA sequences that share more than 25% of their bases do so because they are homologous.

CONCEPT CHECK 22.2

1. Decide whether each of the following pairs of structures more likely represents analogy or homology, and explain your reasoning: (a) a porcupine's quills and a cactus's spines; (b) a cat's paw and a human's hand; (c) an owl's wing and a hornet's wing.
2. **WHAT IF? >** Suppose that two species, A and B, have similar appearances but very divergent gene sequences, while species B and C have very different appearances but similar gene sequences. Which pair of species is more likely to be closely related: A and B or B and C? Explain.

For suggested answers, see Appendix A.

CONCEPT 22.3

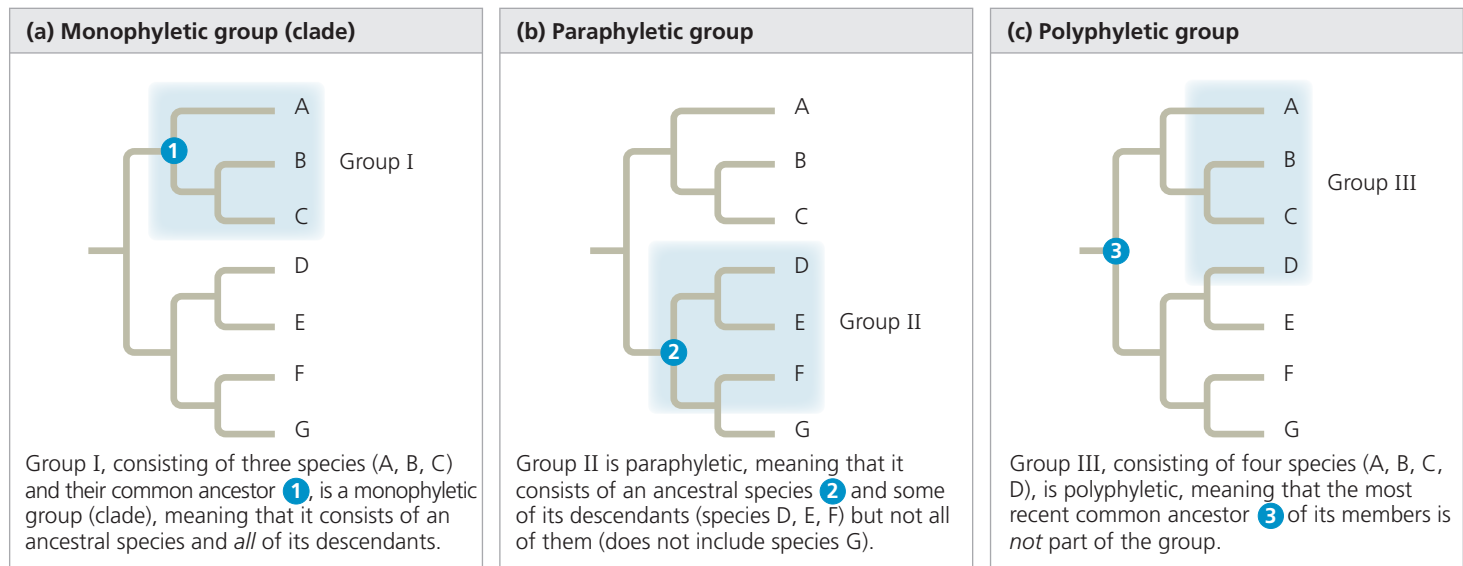
Shared characters are used to construct phylogenetic trees

As we've discussed, a key step in reconstructing phylogenies is to distinguish homologous features from analogous ones (since only homology reflects evolutionary history). Next, we'll describe cladistics, a widely used set of methods for inferring phylogeny from homologous characters.

Cladistics

In the approach to systematics called **cladistics**, common ancestry is the primary criterion used to classify organisms. Using this methodology, biologists attempt to place species into groups called **clades**, each of which includes an ancestral species and all of its descendants. Clades, like taxonomic categories of the Linnaean system, are nested within larger clades. In Figure 22.4, for example, the cat group (Felidae) represents a clade within a larger clade (Carnivora) that also includes the dog group (Canidae).

▼ **Figure 22.10** Monophyletic, paraphyletic, and polyphyletic groups.



However, a taxon is equivalent to a clade only if it is **monophyletic** (from the Greek, meaning “single tribe”), signifying that it consists of an ancestral species and all of its descendants (**Figure 22.10a**). Contrast this with a **paraphyletic** (“beside the tribe”) group, which consists of an ancestral species and some, but not all, of its descendants (**Figure 22.10b**), or a **polyphyletic** (“many tribes”) group, which includes distantly related species but does not include their most recent common ancestor (**Figure 22.10c**).

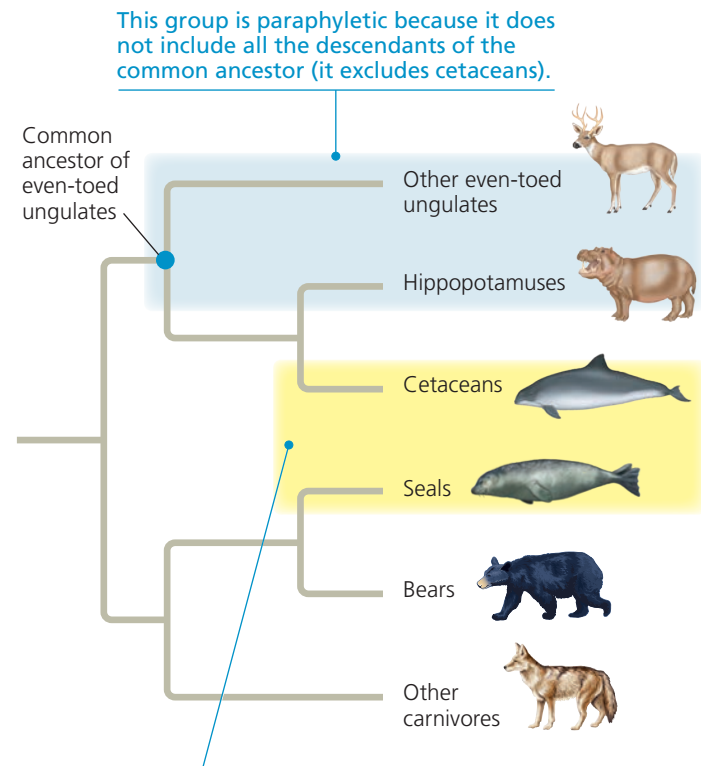
Note that in a paraphyletic group, the most recent common ancestor of all members of the group *is* part of the group, whereas in a polyphyletic group the most recent common ancestor *is not* part of the group. For example, a group consisting of even-toed ungulates (hippopotamuses, deer, and their relatives) and their common ancestor is paraphyletic because it does not include cetaceans (whales, dolphins, and porpoises), which descended from that ancestor (**Figure 22.11**). In contrast, a group consisting of seals and cetaceans (based on their similar body forms) is polyphyletic because it does not include the common ancestor of seals and cetaceans. Biologists avoid defining such polyphyletic groups; if new evidence indicates that an existing group is polyphyletic, its members are reclassified.

Shared Ancestral and Shared Derived Characters

As a result of descent with modification, organisms have characters they share with their ancestors, and they also have characters that differ from those of their ancestors. For example, all mammals have backbones, but a backbone does not distinguish mammals from other vertebrates because *all* vertebrates have backbones. The backbone predates the branching of mammals from other vertebrates. Thus for mammals, the backbone is a **shared ancestral character**, a character that originated in an ancestor of the taxon.

In contrast, hair is a character shared by all mammals but *not* found in their ancestors. Thus, in mammals, hair is considered a **shared derived character**, an evolutionary novelty unique to a clade.

▼ **Figure 22.11** Examples of a paraphyletic and a polyphyletic group.



This group is polyphyletic because it does not include the most recent common ancestor of its members.

DRAW IT ► Circle the branch point that represents the most recent common ancestor of cetaceans and seals. Explain why that ancestor would not be part of a cetacean–seal group defined by their similar body forms.

Note that a shared derived character can refer to the loss of a feature, such as the loss of limbs in snakes or whales. In addition, it is a relative matter whether a character is considered ancestral or derived. A backbone can also qualify as a shared derived character, but only at a deeper branch point that distinguishes all vertebrates from other animals.

Inferring Phylogenies Using Derived Characters

Shared derived characters are unique to particular clades. Because all features of organisms arose at some point in the history of life, it should be possible to determine the clade in which each shared derived character first appeared and to use that information to infer evolutionary relationships.

To give an example of this approach, consider the set of characters shown in **Figure 22.12a** for each of five vertebrates—a leopard, turtle, frog, bass, and lamprey (a jawless aquatic vertebrate). As a basis of comparison, we need to select an outgroup. An **outgroup** is a species or group of species from an evolutionary lineage that is closely related to but not part of the group of species that we are studying (the **ingroup**). A suitable outgroup can be determined based on evidence from morphology, paleontology, embryonic development, and gene sequences. An appropriate outgroup for our example is the lancelet, a small animal that lives in mud-flats and (like vertebrates) is a member of the more inclusive group called the chordates. Unlike the vertebrates, however, the lancelet does not have a backbone.

In our analysis, a character found in both the outgroup and the ingroup is assumed to be ancestral. We'll also assume that each derived character in Figure 22.12a arose only once

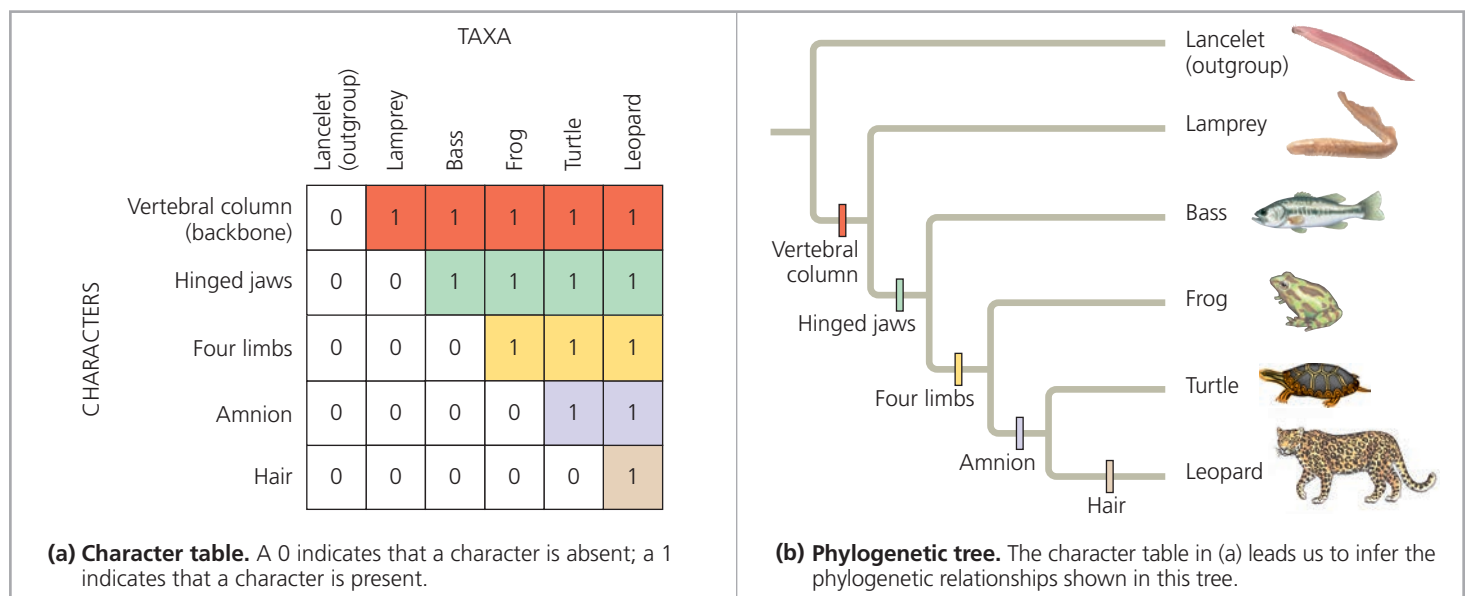
in the ingroup. Thus, for a character that only occurs in a subset of the ingroup, we'll assume that the character arose in the lineage leading to those members of the ingroup.

By comparing members of the ingroup with each other and with the outgroup, we can determine which characters were derived at the various branch points of vertebrate evolution. In our example, *all* of the vertebrates in the ingroup have backbones: This character was present in the ancestral vertebrate, but not in the outgroup. Now note that hinged jaws are absent in the outgroup and in lampreys, but present in all other members of the ingroup. This indicates that hinged jaws arose in a lineage leading to all members of the ingroup *except* lampreys. Hence, we can conclude that lampreys are the sister taxon to the other vertebrates in the ingroup. Proceeding in this way, we can translate the data in our table of characters into a phylogenetic tree that places all the ingroup taxa into a hierarchy based on their shared derived characters (**Figure 22.12b**).

Phylogenetic Trees with Proportional Branch Lengths

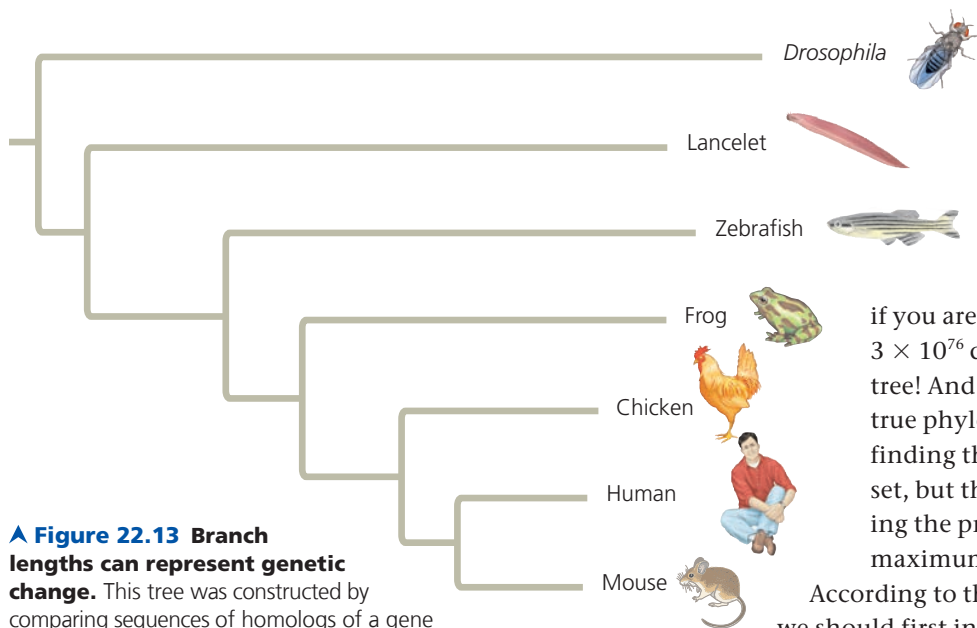
In the phylogenetic trees we have presented so far, the lengths of the tree's branches do not indicate the degree of evolutionary change in each lineage. Furthermore, the chronology represented by the branching pattern of the tree is relative (earlier versus later) rather than absolute (how many millions of years ago). But in some tree diagrams, branch lengths are proportional to amount of evolutionary change or to the times at which particular events occurred.

✓ **Figure 22.12 Using derived characters to infer phylogeny.** The derived characters used here include the amnion, a membrane that encloses the embryo inside a fluid-filled sac (see Figure 34.26). Note that a different set of characters could lead us to infer a different phylogenetic tree.



DRAW IT ▶ In (b), circle the most inclusive clade for which a hinged jaw is a shared ancestral character.

Figure Walkthrough



▲ Figure 22.13 Branch lengths can represent genetic change. This tree was constructed by comparing sequences of homologs of a gene that plays a role in development; *Drosophila* was used as an outgroup. The branch lengths are proportional to the amount of genetic change in each lineage; varying branch lengths indicate that the gene has evolved at different rates in different lineages.

INTERPRET THE DATA ▶ In which vertebrate lineage has the studied gene evolved most rapidly? Explain.

In **Figure 22.13**, for example, the branch length of the phylogenetic tree reflects the number of changes that have taken place in a particular DNA sequence in that lineage. Note that the total length of the horizontal lines from the base of the tree to the mouse is less than that of the line leading to the outgroup species, the fruit fly *Drosophila*. This implies that in the time since the mouse and fly lineages diverged from their common ancestor, more genetic changes have occurred in the *Drosophila* lineage than in the mouse lineage.

Even though the branches of a phylogenetic tree may have different lengths, among organisms alive today, all the different lineages that descend from a common ancestor have survived for the same number of years. To take an extreme example, humans and bacteria had a common ancestor that lived over 3 billion years ago. Fossils and genetic evidence indicate that this ancestor was a single-celled prokaryote. Even though bacteria have apparently changed little in their morphology since that common ancestor, there have nonetheless been 3 billion years of evolution in the bacterial lineage, just as there have been 3 billion years of evolution in the lineage that ultimately gave rise to humans.

These equal spans of chronological time can be represented in a phylogenetic tree whose branch lengths are proportional to time (**Figure 22.14**). Such a tree draws on fossil data to place branch points in the context of geologic time. Additionally, it is possible to combine these two types of trees by labeling branch points with information about rates of genetic change or dates of divergence.

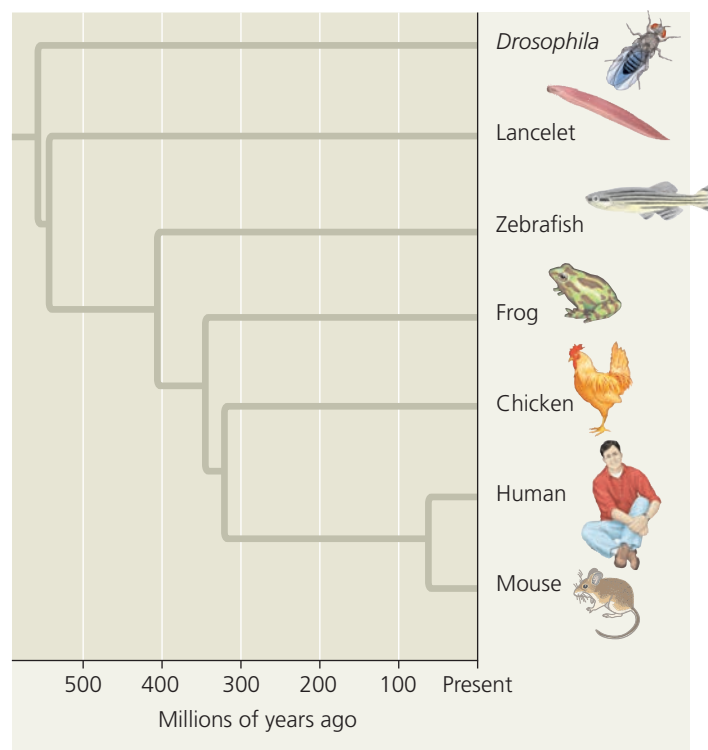
Maximum Parsimony and Maximum Likelihood

As the database of DNA sequences that enables us to study more species grows, the difficulty of building the phylogenetic tree that best describes their evolutionary history also grows. What

if you are analyzing data for 50 species? There are 3×10^{76} different ways to arrange 50 species into a tree! And which tree in this huge forest reflects the true phylogeny? Systematists can never be sure of finding the most accurate tree in such a large data set, but they can narrow the possibilities by applying the principles of maximum parsimony and maximum likelihood.

According to the principle of **maximum parsimony**, we should first investigate the simplest explanation that is consistent with the facts. (The parsimony principle is also called “Occam’s razor” after William of Occam, a 14th-century English philosopher who advocated this minimalist problem-solving approach of “shaving away” unnecessary complications.) In the case of trees based on morphology, the most parsimonious tree requires the fewest evolutionary events, as measured by the origin of shared

▼ Figure 22.14 Branch lengths can indicate time. This tree is based on the same DNA data as that in Figure 22.13, but here the branch points are dated based on fossil evidence. Thus, the branch lengths are proportional to time. Each lineage has the same total length from the base of the tree to the branch tip, indicating that all the lineages have diverged from the common ancestor for equal amounts of time.



derived morphological characters. For phylogenies based on DNA, the most parsimonious tree requires the fewest base changes.

A **maximum likelihood** approach identifies the tree most likely to have produced a given set of DNA data, based on certain probability rules about how DNA sequences change over time. For example, the underlying probability rules could be based on the assumption that all nucleotide substitutions are equally likely. However, if evidence suggests

that this assumption is not correct, more complex rules could be devised to account for different rates of change among different nucleotides or at different positions in a gene.

Scientists have developed many computer programs to search for trees that are parsimonious and likely. When a large amount of accurate data is available, the methods used in these programs usually yield similar trees. As an example of one method, **Figure 22.15** walks you through the process of identifying the most parsimonious molecular tree for

▼ Figure 22.15

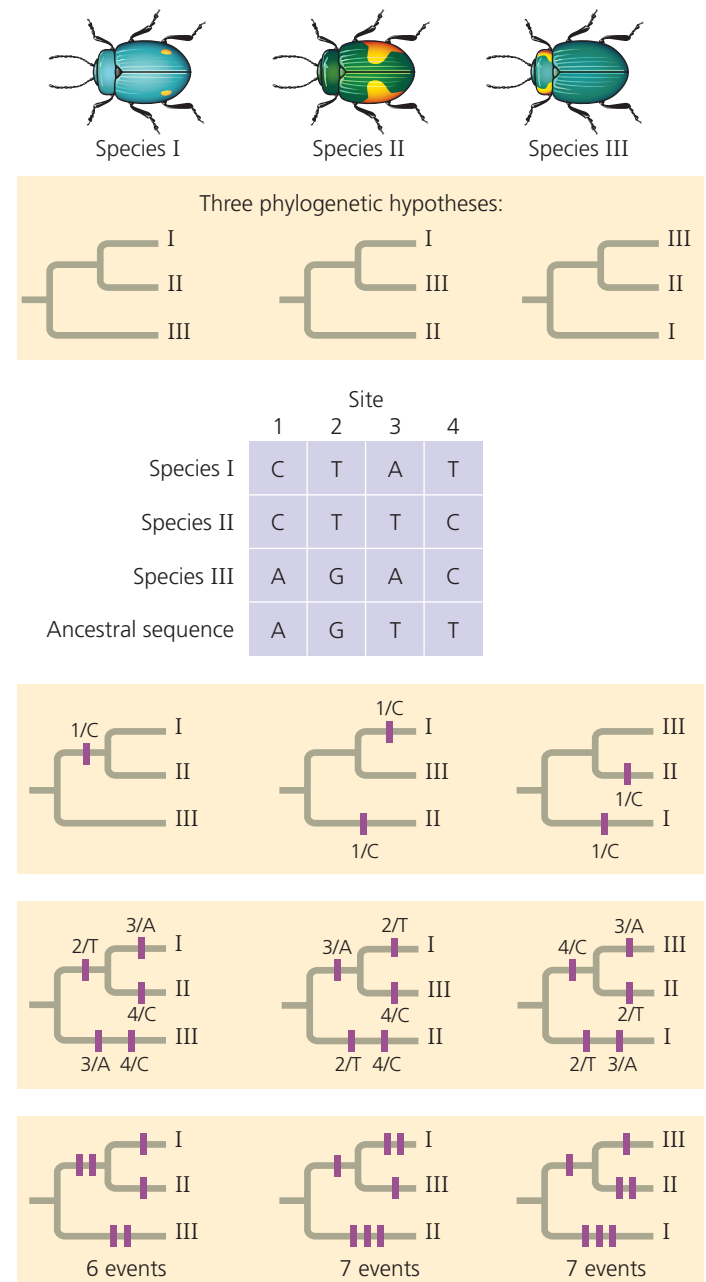
Research Method Applying Parsimony to a Problem in Molecular Systematics

Application In considering possible phylogenies for a group of species, systematists compare molecular data for the species. An efficient way to begin is by identifying the most parsimonious hypothesis—the one that requires the fewest evolutionary events (molecular changes) to have occurred.

Technique Follow the numbered steps as we apply the principle of parsimony to a hypothetical phylogenetic problem involving three closely related beetle species.

- 1 First, draw the three possible trees for the species. (Although only 3 trees are possible when ordering 3 species, the number of possible trees increases rapidly with the number of species: There are 15 trees for 4 species and 34,459,425 trees for 10 species.)
- 2 Tabulate the molecular data for the species. In this simplified example, the data represent a DNA sequence consisting of just four nucleotide bases. Data from several outgroup species (not shown) were used to infer the ancestral DNA sequence.
- 3 Now focus on site 1 in the DNA sequence. In the tree on the left, a single base-change event, represented by the purple hatch mark on the branch leading to species I and II (and labeled 1/C, indicating a change at site 1 to nucleotide C), is sufficient to account for the site 1 data. In the other two trees, two base-change events are necessary.
- 4 Continuing the comparison of bases at sites 2, 3, and 4 reveals that each of the three trees requires a total of five additional base-change events (purple hatch marks).

Results To identify the most parsimonious tree, we total all of the base-change events noted in steps 3 and 4. We conclude that the first tree is the most parsimonious of the three possible phylogenies. (In a real example, many more sites would be analyzed. Hence, the trees would often differ by more than one base-change event.)



a three-species problem. Computer programs use the principle of parsimony to estimate phylogenies in a similar way: They examine large numbers of possible trees and identify those that require the fewest evolutionary changes.

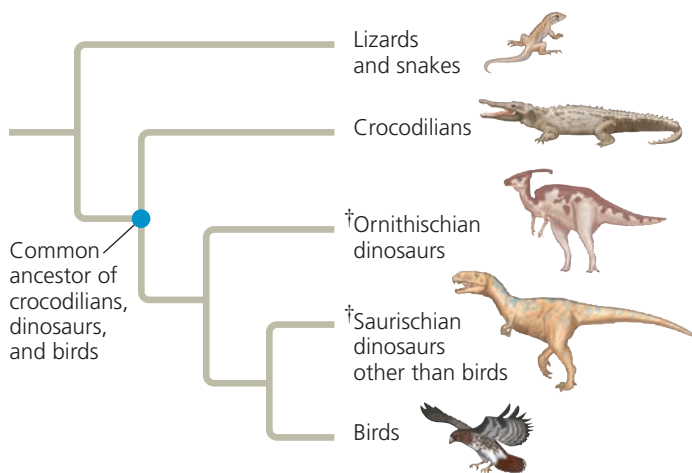
Phylogenetic Trees as Hypotheses

This is a good place to reiterate that any phylogenetic tree represents a hypothesis about how the organisms in the tree are related to one another. The best hypothesis is the one that best fits all the available data. A phylogenetic hypothesis may be modified when new evidence compels systematists to revise their trees. Indeed, while many older phylogenetic hypotheses have been supported by new morphological and molecular data, others have been changed or rejected.

Thinking of phylogenies as hypotheses also allows us to use them in a powerful way: We can make and test predictions based on the assumption that a particular phylogeny—our hypothesis—is correct. For example, in an approach known as *phylogenetic bracketing*, we can predict (by parsimony) that features shared by two groups of closely related organisms are present in their common ancestor and all of its descendants unless independent data indicate otherwise. (Note that “prediction” can refer to unknown past events as well as to evolutionary changes yet to occur.)

This approach has been used to make novel predictions about dinosaurs. For example, there is evidence that birds descended from the theropods, a group of bipedal saurischian dinosaurs. As seen in **Figure 22.16**, the closest living relatives of birds are crocodiles. Birds and crocodiles share numerous features: They have four-chambered hearts, they “sing” to defend territories and attract mates (although a crocodile’s “song” is more like a bellow), and they build nests. Both birds and crocodiles also care for their eggs by *brooding*, a behavior

▼ **Figure 22.16** A phylogenetic tree of birds and their close relatives. († indicates extinct lineages.)

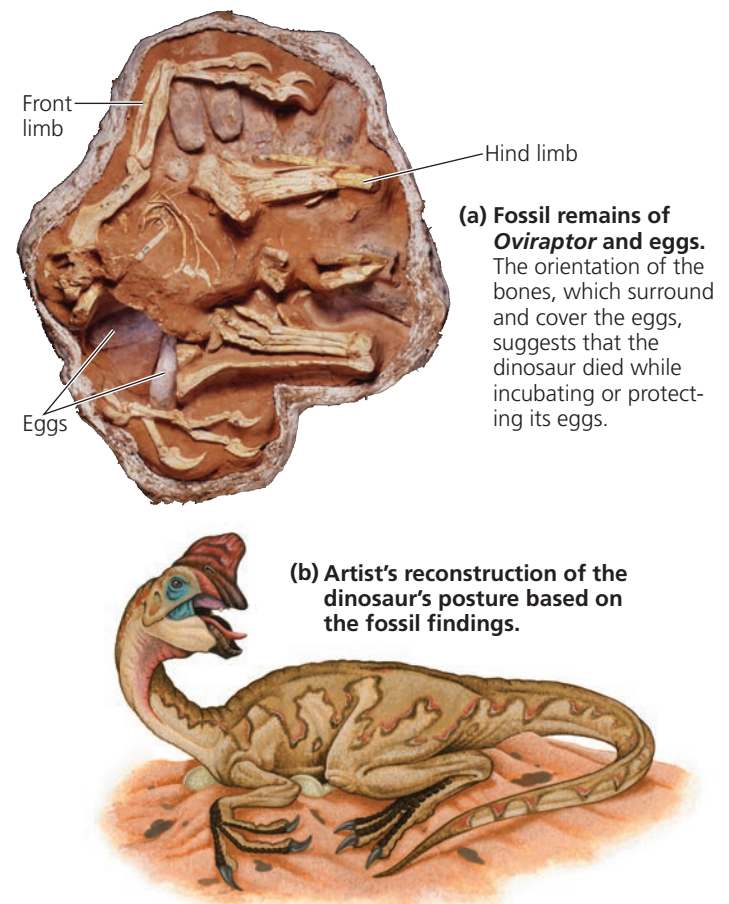


VISUAL SKILLS ► In this tree diagram, what is the sister taxon of the clade that includes dinosaurs and their most recent common ancestor? Explain.

in which a parent warms the eggs with its body. Birds brood by sitting on their eggs, whereas crocodiles cover their eggs with their neck. Reasoning that any feature shared by birds and crocodiles is likely to have been present in their common ancestor (denoted by the blue dot in Figure 22.16) and *all* of its descendants, biologists predicted that dinosaurs had four-chambered hearts, sang, built nests, and exhibited brooding.

Internal organs, such as the heart, rarely fossilize, and it is, of course, difficult to test whether dinosaurs sang to defend territories and attract mates. However, fossilized dinosaur eggs and nests have provided evidence supporting the prediction of brooding in dinosaurs. First, a fossil embryo of an *Oviraptor* dinosaur was found, still inside its egg. This egg was identical to those found in another fossil, one that showed an *Oviraptor* crouching over a group of eggs in a posture similar to that seen in brooding birds today (**Figure 22.17**). Researchers suggested that the *Oviraptor* dinosaur preserved in this second fossil died while incubating or protecting its eggs. The broader conclusion that emerged from this work—that dinosaurs built nests and exhibited brooding—has since been strengthened by additional fossil discoveries that show that other species of dinosaurs built nests and sat on their eggs. Finally, by supporting predictions based on the phylogenetic

▼ **Figure 22.17** Fossil support for a phylogenetic prediction: Dinosaurs built nests and brooded their eggs.



hypothesis shown in Figure 22.16, fossil discoveries of nests and brooding in dinosaurs provide independent data that suggest that the hypothesis is correct.

CONCEPT CHECK 22.3

1. To distinguish a particular clade of mammals within the larger clade that corresponds to class Mammalia, would hair be a useful character? Why or why not?
2. The most parsimonious tree of evolutionary relationships can be inaccurate. How can this occur?
3. **WHAT IF? >** Draw a phylogenetic tree that includes the relationships from Figure 25.7 and Figure 22.16. Traditionally, all the taxa shown besides birds and mammals were classified as reptiles. Would a cladistic approach support that classification? Explain.

For suggested answers, see Appendix A.

CONCEPT 22.4

An organism's evolutionary history is documented in its genome

As you have seen in this chapter, comparisons of nucleic acids or other molecules can be used to deduce relatedness. In some cases, such comparisons can reveal phylogenetic relationships that cannot be determined by nonmolecular methods such as comparative anatomy. For example, the analysis of molecular data helps us uncover evolutionary relationships between groups that have little common ground for morphological comparison, such as animals and fungi. And molecular methods allow us to reconstruct phylogenies among groups of present-day organisms for which the fossil record is poor or lacking entirely.

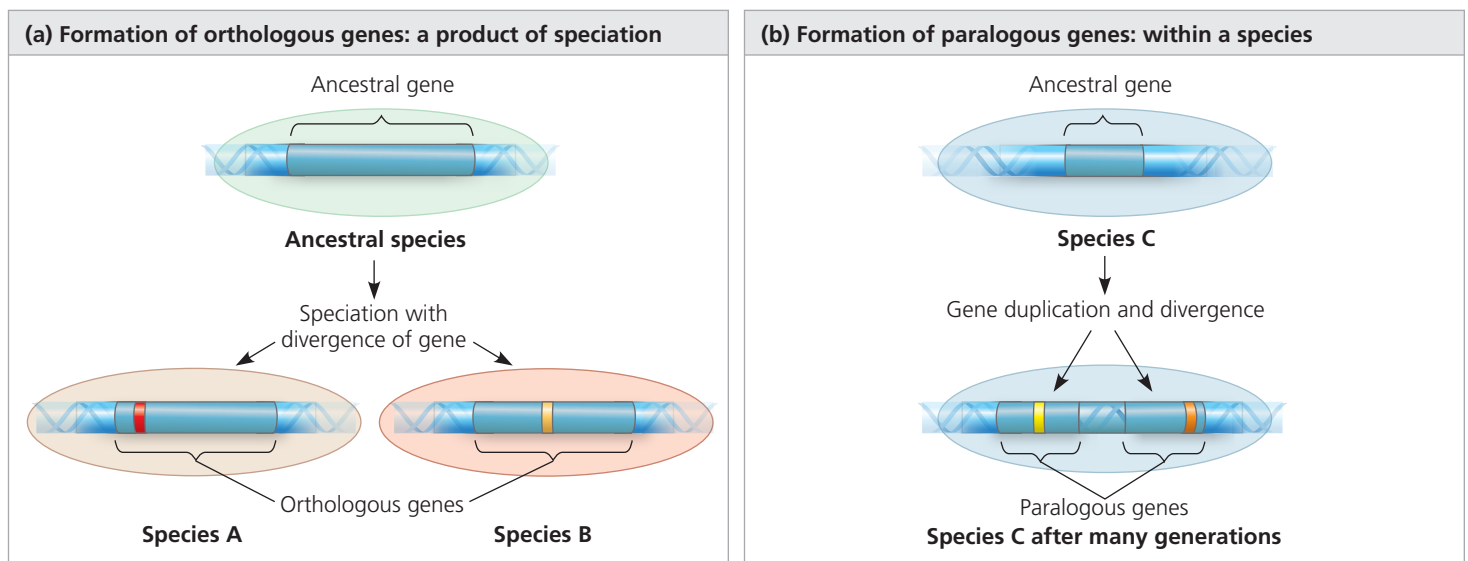
Different genes can evolve at different rates, even in the same evolutionary lineage. As a result, molecular trees can represent short or long periods of time, depending on which genes are used. For example, the DNA that codes for ribosomal RNA (rRNA) changes relatively slowly. Therefore, comparisons of DNA sequences in these genes are useful for investigating relationships between taxa that diverged hundreds of millions of years ago. Studies of rRNA sequences indicate, for instance, that fungi are more closely related to animals than to plants. In contrast, mitochondrial DNA (mtDNA) evolves relatively rapidly and can be used to explore recent evolutionary events. One research team has traced the relationships among Native American groups through their mtDNA sequences. The molecular findings corroborate other evidence that the Pima of Arizona, the Maya of Mexico, and the Yanomami of Venezuela are closely related, probably descending from the first of three waves of immigrants that crossed the Bering Land Bridge from Asia to the Americas about 15,000 years ago.

Gene Duplications and Gene Families

What do molecular data reveal about the evolutionary history of genome change? Consider gene duplication, which plays an important role in evolution because it increases the number of genes in the genome, providing more opportunities for further evolutionary changes. Molecular techniques now allow us to trace the phylogenies of gene duplications. These molecular phylogenies must account for repeated duplications that have resulted in *gene families*, groups of related genes within an organism's genome (see Figure 20.11).

Accounting for such duplications leads us to distinguish two types of homologous genes (**Figure 22.18**): orthologous genes and paralogous genes. In **orthologous genes**

▼ **Figure 22.18 Two types of homologous genes.** Colored bands mark regions of the genes where differences in base sequences have accumulated.



(from the Greek *orthos*, exact), the homology results from speciation (the splitting of one species into two or more species) and hence occurs between genes found in different species (see Figure 22.18a). For example, the genes that code for cytochrome *c* (a protein that functions in electron transport chains) in humans and dogs are orthologous. In **paralogous genes** (from the Greek *para*, in parallel), the homology results from gene duplication; hence, multiple copies of these genes have diverged from one another within a species (see Figure 22.18b). In Concept 23.1, you encountered the example of olfactory receptor genes, which have undergone many gene duplications in vertebrates; humans have 380 functional copies of these paralogous genes, while mice have 1,200.

Note that orthologous genes can diverge only after speciation has taken place, that is, after the genes are found in the genomes of different species. For example, although the cytochrome *c* genes in humans and dogs serve the same function, the gene's sequence in humans has diverged from that in dogs in the time since these species last shared a common ancestor. Paralogous genes, on the other hand, can diverge within a species because they are present in more than one copy in the genome. The paralogous genes that make up the olfactory receptor gene family in humans have diverged from each other during our long evolutionary history. They now specify proteins that confer sensitivity to a wide variety of molecules, ranging from food odors to sex pheromones.

Genome Evolution

Now that we can compare the entire genomes of different organisms, including our own, two patterns have emerged. First, lineages that diverged long ago often share many orthologous genes. For example, though the human and mouse lineages diverged about 65 million years ago, 99% of the genes of humans and mice are orthologous. And 50% of human genes are orthologous with those of yeast, despite 1 billion years of divergent evolution. Such commonalities explain why disparate organisms nevertheless share many biochemical and developmental pathways. As a result of these shared pathways, the functioning of genes linked to diseases in humans can often be investigated by studying yeast and other organisms distantly related to humans.

Second, the number of genes a species has doesn't seem to increase through duplication at the same rate as perceived phenotypic complexity. Humans have only about four times as many genes as yeast, a single-celled eukaryote, even though—unlike yeast—we have a large, complex brain and a body with more than 200 different types of tissues. Evidence is emerging that many human genes are more versatile than those of yeast: A single human gene can encode multiple proteins that perform different tasks in various body tissues. Unraveling the mechanisms that cause this genomic versatility and phenotypic variation is an exciting challenge.

CONCEPT CHECK 22.4

1. Explain how comparing proteins of two species can yield data about the species' evolutionary relationship.
2. **WHAT IF? >** Suppose gene A is orthologous in species 1 and species 2, and gene B is paralogous to gene A in species 1. Suggest a sequence of two evolutionary events that could result in the following: Gene A differs considerably between species, yet gene A and gene B show little divergence from each other.
3. **MAKE CONNECTIONS >** Review Figure 18.13, then suggest how a particular gene could have different functions in different tissues within an organism.

For suggested answers, see Appendix A.

CONCEPT 22.5

Molecular clocks help track evolutionary time

One goal of evolutionary biology is to understand the relationships among all organisms. It is also helpful to know when lineages diverged from one another, including those for which there is no fossil record. But how can we determine the timing of phylogenies that extend beyond the fossil record?

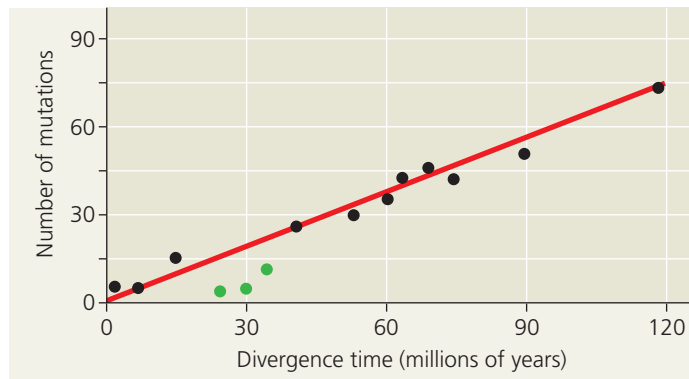
Molecular Clocks

We stated earlier that researchers have estimated that the common ancestor of Hawaiian silversword plants lived about 5 million years ago. How did they make this estimate? They relied on the concept of a **molecular clock**, an approach for measuring the absolute time of evolutionary change based on the observation that some genes and other regions of genomes appear to evolve at constant rates. An assumption underlying the molecular clock is that the number of nucleotide substitutions in orthologous genes is proportional to the time that has elapsed since the genes branched from their common ancestor. In the case of paralogous genes, the number of substitutions is proportional to the time since the ancestral gene was duplicated.

We can calibrate the molecular clock of a gene that has a reliable average rate of evolution by graphing the number of genetic differences—for example, nucleotide, codon, or amino acid differences—against the dates of evolutionary branch points that are known from the fossil record (**Figure 22.19**). The average rates of genetic change inferred from such graphs can then be used to estimate the dates of events that cannot be discerned from the fossil record, such as the origin of the silverswords discussed earlier.

Of course, no gene marks time with complete precision. In fact, some portions of the genome appear to have evolved in irregular bursts that are not at all clocklike. And even those genes that seem to act as reliable molecular clocks

▼ **Figure 22.19 A molecular clock for mammals.** The number of accumulated mutations in seven proteins has increased over time in a consistent manner for most mammal species. The three green data points represent primate species, whose proteins appear to have evolved more slowly than those of other mammals. The divergence time for each data point was based on fossil evidence.



INTERPRET THE DATA ► Use the graph to estimate the divergence time for a mammal with a total of 30 mutations in the seven proteins.

are accurate only in the statistical sense of showing a fairly smooth *average* rate of change. Over time, there may still be deviations from that average rate. Furthermore, the same gene may evolve at different rates in different groups of organisms. Finally, when comparing genes that are clock-like, the rate of the clock may vary greatly from one gene to another; some genes evolve a million times faster than others.

Differences in Clock Speed

What causes such differences in the speed at which clock-like genes evolve? The answer stems from the fact that some mutations are selectively neutral—neither beneficial nor detrimental. Of course, many new mutations are harmful and are removed quickly by selection. But if most of the rest are neutral and have little or no effect on fitness, then the rate of evolution of those neutral mutations should indeed be regular, like a clock. Differences in the clock rate for different genes are related to how important a gene is. If the exact sequence of amino acids that a gene specifies is essential to survival, most of the mutational changes will be harmful and only a few will be neutral. As a result, such genes change only slowly. But if the exact sequence of amino acids is less critical, fewer of the new mutations will be harmful and more will be neutral. Such genes change more quickly.

Potential Problems with Molecular Clocks

As we've seen, molecular clocks do not run as smoothly as would be expected if the underlying mutations were selectively neutral. Many irregularities are likely to be the result of natural selection in which certain DNA changes are

favored over others. Indeed, evidence suggests that almost half the amino acid differences in proteins of two *Drosophila* species, *D. simulans* and *D. yakuba*, are not neutral but have resulted from natural selection. But because the direction of natural selection may change repeatedly over long periods of time (and hence may average out), some genes experiencing selection can nevertheless serve as approximate markers of elapsed time.

Another question arises when researchers attempt to extend molecular clocks beyond the time span documented by the fossil record. Although an abundant fossil record extends back only about 550 million years, molecular clocks have been used to date evolutionary divergences that occurred a billion or more years ago. These estimates assume that the clocks have been constant for all that time. Such estimates are highly uncertain.

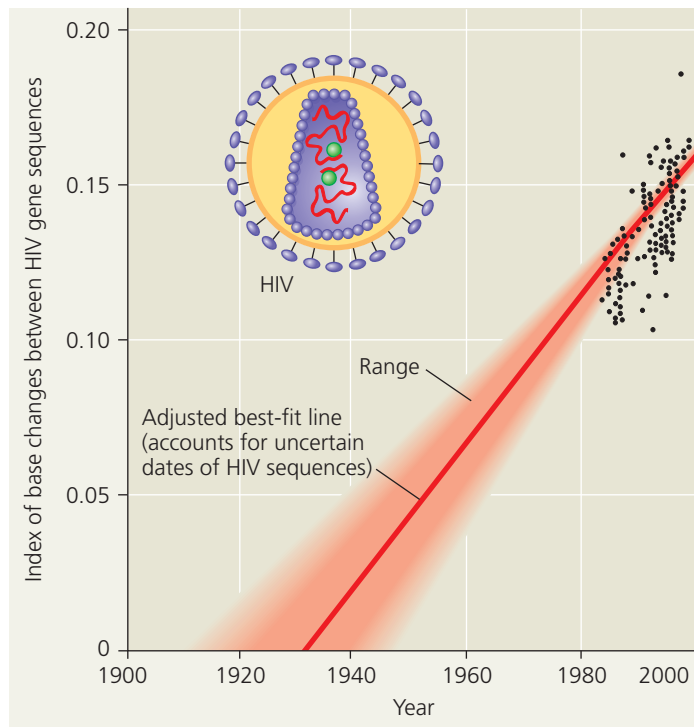
In some cases, problems may be avoided by calibrating molecular clocks with data on the rates at which genes have evolved in different taxa. In other cases, problems may be avoided by using many genes rather than just using one or a few genes. By using many genes, fluctuations in evolutionary rate due to natural selection or other factors that vary over time may average out. For example, one group of researchers constructed molecular clocks of vertebrate evolution from published sequence data for 658 nuclear genes. Despite the broad period of time covered (nearly 600 million years) and the fact that natural selection probably affected some of these genes, their estimates of divergence times agreed closely with fossil-based estimates. As this example suggests, if used with care, molecular clocks can aid our understanding of evolutionary relationships.

Applying a Molecular Clock: Dating the Origin of HIV

Researchers have used a molecular clock to date the origin of HIV infection in humans. Phylogenetic analysis shows that HIV, the virus that causes AIDS, is descended from viruses that infect chimpanzees and other primates. (Most of these viruses do not cause AIDS-like diseases in their native hosts.) When did HIV jump to humans? There is no simple answer because the virus has spread to humans more than once. The multiple origins of HIV are reflected in the variety of strains (genetic types) of the virus. HIV's genetic material is made of RNA, and like other RNA viruses, it evolves quickly.

The most widespread strain in humans is HIV-1 M. To pinpoint the earliest HIV-1 M infection, researchers compared samples of the virus from various times during the epidemic, including a sample from 1959. A comparison of gene sequences showed that the virus has evolved in a clocklike fashion. Extrapolating backward in time using the molecular

▼ **Figure 22.20 Dating the origin of HIV-1 M.** The black data points are based on DNA sequences of an HIV gene in patients' blood samples. (The dates when these individual HIV gene sequences arose are not certain because a person can harbor the virus for years before symptoms occur.) Projecting the gene's rate of change backward in time by this method suggests that the virus originated in the 1930s.



clock indicates that the HIV-1 M strain first spread to humans around 1930 (**Figure 22.20**). A later study, which dated the origin of HIV using a more advanced molecular clock approach than that covered in this book, estimated that the HIV-1 M strain first spread to humans around 1910.

CONCEPT CHECK 22.5

1. What is a molecular clock? What assumption underlies the use of a molecular clock?
2. **MAKE CONNECTIONS** > Review Concept 17.5. Then explain how numerous base changes could occur in an organism's DNA yet have no effect on its fitness.
3. **WHAT IF?** > Suppose a molecular clock dates the divergence of two taxa at 80 million years ago, but new fossil evidence shows that the taxa diverged at least 120 million years ago. Explain how this could happen.

For suggested answers, see Appendix A.

CONCEPT 22.6

Our understanding of the tree of life continues to change based on new data

The discovery that the glass lizard in **Figure 22.1** evolved from a different lineage of legless lizards than did snakes is one example of how our understanding of life's diversity is

informed by systematics. Indeed, in recent decades, systematists have gained insight into even the very deepest branches of the tree of life by analyzing DNA sequence data.

From Two Kingdoms to Three Domains

Taxonomists once classified all known species into two kingdoms: plants and animals. Classification schemes with more than two kingdoms gained broad acceptance in the late 1960s, when many biologists recognized five kingdoms: Monera (prokaryotes), Protista (a diverse kingdom consisting mostly of unicellular organisms), Plantae, Fungi, and Animalia. This system highlighted the two fundamentally different types of cells, prokaryotic and eukaryotic, and set the prokaryotes apart from all eukaryotes by placing them in their own kingdom, Monera.

However, phylogenies based on genetic data soon revealed a problem with this system: Some prokaryotes differ as much from each other as they do from eukaryotes. Such difficulties led biologists to adopt a three-domain system. The three domains—Bacteria, Archaea, and Eukarya—are a taxonomic level higher than the kingdom level. The validity of these domains has been supported by many studies, including a recent study that analyzed nearly 100 completely sequenced genomes.

The domain Bacteria contains most of the currently known prokaryotes, while the domain Archaea consists of a diverse group of prokaryotic organisms that inhabit a wide variety of environments. The domain Eukarya consists of all the organisms that have cells containing true nuclei. This domain includes many groups of single-celled organisms as well as multicellular plants, fungi, and animals. **Figure 22.21** represents one possible phylogenetic tree for the three domains and some of the many lineages they encompass.

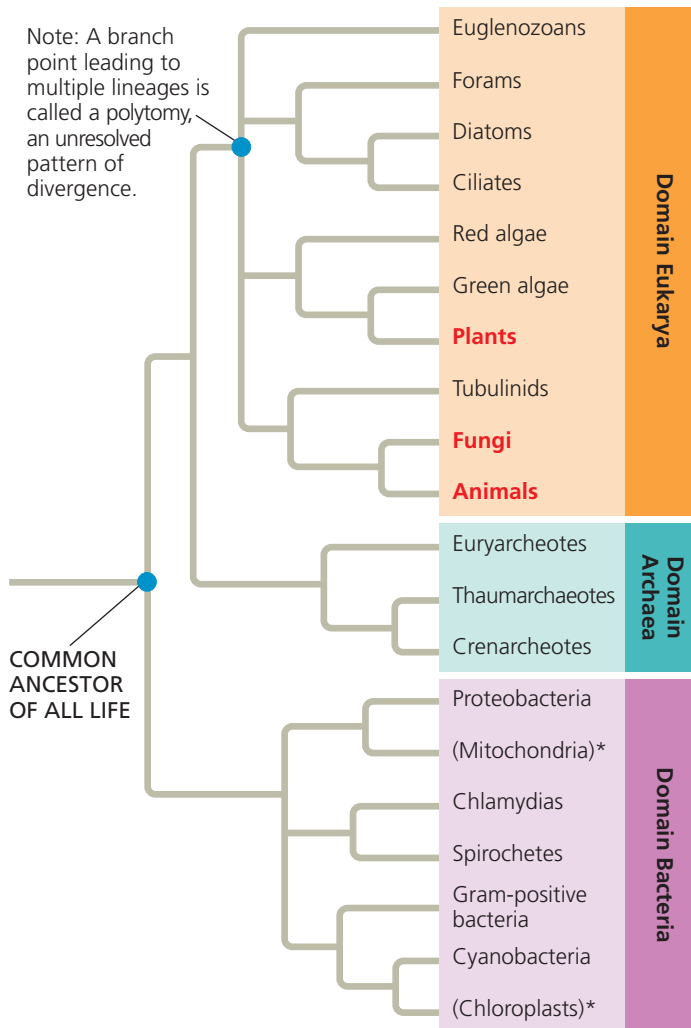
The three-domain system highlights the fact that much of the history of life has been about single-celled organisms. The two prokaryotic domains consist entirely of single-celled organisms, and even in Eukarya, only the branches labeled in red type (plants, fungi, and animals) are dominated by multicellular organisms. Of the five kingdoms previously recognized by taxonomists, most biologists continue to recognize Plantae, Fungi, and Animalia, but not Monera and Protista. The kingdom Monera is obsolete because it would have members in two different domains. The kingdom Protista has also crumbled because it includes members that are more closely related to plants, fungi, or animals than to other protists (see **Figure 28.2**).

New research continues to change our understanding of the tree of life. For example, in the past decade, metagenomic studies have uncovered the genomes of many new species of archaea, leading to discovery of the Thaumarchaeota and other previously unknown phyla of archaea (see **Concept 27.4**).

The Important Role of Horizontal Gene Transfer

In the phylogeny shown in **Figure 22.21**, the first major split in the history of life occurred when bacteria diverged from

▼ **Figure 22.21 The three domains of life.** This phylogenetic tree is based on sequence data for rRNA and other genes. For simplicity, only some of the major branches in each domain are shown. Lineages within Eukarya that are dominated by multicellular organisms (plants, fungi, and animals) are in red type, while the two lineages denoted by an asterisk are based on DNA from cellular organelles. All other lineages consist solely or mainly of single-celled organisms.



MAKE CONNECTIONS ► After reviewing endosymbiont theory (see Figure 7.16), explain the specific positions of the mitochondrion and chloroplast lineages on this tree.

other organisms. If this tree is correct, eukaryotes and archaea are more closely related to each other than either is to bacteria.

This reconstruction of the tree of life is based in part on sequence comparisons of rRNA genes, which code for the RNA components of ribosomes. However, some other genes reveal a different set of relationships. For example, researchers have found that many of the genes that influence metabolism in yeast (a unicellular eukaryote) are more similar to genes in the domain Bacteria than they are to genes in the domain Archaea—a finding that suggests that the eukaryotes may share a more recent common ancestor with bacteria than with archaea.

What causes trees based on data from different genes to yield such different results? Comparisons of complete genomes from the three domains show that there have been substantial

movements of genes between organisms in the different domains. These took place through **horizontal gene transfer**, a process in which genes are transferred from one genome to another through mechanisms such as exchange of transposable elements and plasmids, viral infection (see Concept 26.2), and perhaps fusions of organisms (as when a host and its endosymbiont become a single organism). Recent research reinforces the view that horizontal gene transfer is important. For example, one study found that on average, 80% of the genes in 181 prokaryotic genomes had moved between species at some point during the course of evolution. Because phylogenetic trees are based on the assumption that genes are passed vertically from one generation to the next, the occurrence of such horizontal transfer events helps to explain why trees built using different genes can give inconsistent results.

Horizontal gene transfer can also occur between eukaryotes. For example, over 200 cases of the horizontal transfer of transposons have been reported in eukaryotes, including humans and other primates, plants, birds, and lizards. Nuclear genes have also been transferred horizontally from one eukaryote to another. The **Scientific Skills Exercise** describes one such example, giving you the opportunity to interpret data collected by Nancy Moran on the transfer of a pigment gene to an aphid from another species.

Recent evidence indicates that eukaryotes can even acquire nuclear genes from bacteria and archaea. For example, a 2013 genomic analysis showed that the alga *Galdieria sulphuraria* (Figure 22.22) acquired about 5% of its genes from various bacterial and archaeal species. Unlike most eukaryotes, this alga can survive in environments that are highly acidic or extremely hot, as well as those with high concentrations of heavy metals. The researchers identified specific genes transferred from prokaryotes that have enabled *G. sulphuraria* to thrive in such extreme habitats.

Overall, horizontal gene transfer has played a key role throughout the evolutionary history of life, and it continues to occur today. Some biologists have argued that horizontal gene transfer was so common that the early history of life should be represented not as a dichotomously branching tree

▼ **Figure 22.22 A recipient of transferred genes: the alga *Galdieria sulphuraria*.** Genes received from prokaryotes enable *G. sulphuraria* (inset) to grow in extreme environments, including on sulfur-encrusted rocks around volcanic hot springs similar to this one in Yellowstone National Park.



SCIENTIFIC SKILLS EXERCISE

Using Protein Sequence Data to Test an Evolutionary Hypothesis

Did Aphids Acquire Their Ability to Make Carotenoids Through Horizontal Gene Transfer? Carotenoids are colored molecules that have diverse functions in many organisms, such as photosynthesis in plants and light detection in animals. Plants and many microorganisms can synthesize carotenoids from scratch, but animals generally cannot (they must obtain carotenoids from their diet). One exception is the pea aphid *Acyrtosiphon pisum*, a small, plant-dwelling insect whose genome includes a full set of genes for the enzymes needed to make carotenoids. Because other animals lack these genes, it is unlikely that aphids inherited them from a single-celled common ancestor shared with microorganisms and plants. So where did they come from? Evolutionary biologists hypothesized that an aphid ancestor acquired these genes by horizontal gene transfer from distantly related organisms.

How the Experiment Was Done Scientists obtained the DNA sequences for the carotenoid-biosynthesis genes from several species, including aphids, fungi, bacteria, and plants. A computer “translated” these sequences into amino acid sequences of the encoded polypeptides and aligned the amino acid sequences. This allowed the team to compare the corresponding polypeptides in the different organisms.

Data from the Experiment The sequences below show the first 60 amino acids of one polypeptide of the carotenoid-biosynthesis enzymes in the plant *Arabidopsis thaliana* (bottom) and the corresponding amino acids in five nonplant species, using the one-letter

abbreviations for the amino acids (see Figure 5.14). A dash (–) indicates a gap inserted in a sequence to optimize its alignment with the corresponding sequence in *Arabidopsis*.



INTERPRET THE DATA

1. In the rows of data for the organisms being compared with the aphid, highlight the amino acids that are identical to the corresponding amino acids in the aphid.
2. Which organism has the most amino acids in common with the aphid? Rank the partial polypeptides from the other four organisms in degree of similarity to that of the aphid.
3. Do these data support the hypothesis that aphids acquired the gene for this polypeptide by horizontal gene transfer? Why or why not? If horizontal gene transfer did occur, what type of organism is likely to have been the source?
4. What additional sequence data would support your hypothesis?
5. How would you account for the similarities between the aphid sequence and the sequences for the bacteria and plant?



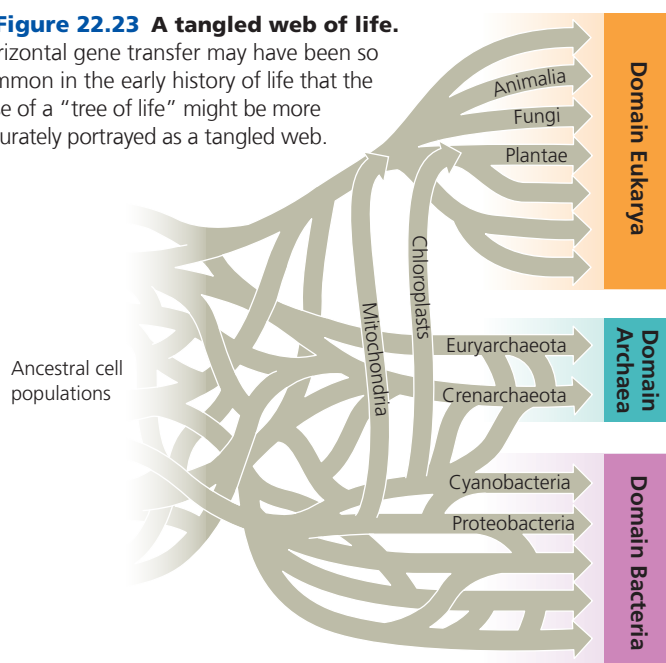
Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Data from Nancy A. Moran, Yale University. See N. A. Moran and T. Jarvik, Lateral transfer of genes from fungi underlies carotenoid production in aphids, *Science* 328:624–627 (2010).

Organism	Alignment of Amino Acid Sequences
<i>Acyrtosiphon</i> (aphid)	IKIIIGSGV GGTAAARLS KKGFGVEVYE KNSYNGGRCS IIR-HNGHRF DQGPSL--YL
<i>Ustilago</i> (fungus)	KKVVIIGAGA GTTALAARLG RRGYSVTVLE KNSFGGGRCS LIH-HDGHRW DQGPSL--YL
<i>Gibberella</i> (fungus)	KSVIVIGAGV GGVSTAARLA KAGFKVTILE KNDFTGGRCS LIH-NDGHRF DQGPSL--LL
<i>Staphylococcus</i> (bacterium)	MKIAVIGAGV TGLAAAARIA SQGHEVTIFE KNNVGGGRMN QLK-KDGFTF DMGPTI--VM
<i>Pantoea</i> (bacterium)	KRTFVIGAGF GGLALAIRLQ AAGIATTVLE QHDKPGGRAY VWQ-DQGFTF DAGPTV--IT
<i>Arabidopsis</i> (plant)	WDAVVIGGGH NGLTAAAYLA RGGLSVAVLE RRHVIGGAHV TEEIVPGFKF SRCSYLQGLL

► Figure 22.23 A tangled web of life.

Horizontal gene transfer may have been so common in the early history of life that the base of a “tree of life” might be more accurately portrayed as a tangled web.



like that in Figure 22.21, but rather as a tangled network of connected branches (Figure 22.23). Although scientists continue to debate how best to portray the earliest steps in the history of life, in recent decades there have been many exciting discoveries about evolutionary events that occurred over time. We’ll explore the mechanisms that underlie such events in the rest of this unit’s chapters, beginning with the evolution of populations.

CONCEPT CHECK 22.6

1. Why is the kingdom Monera no longer considered a valid taxon?
2. Explain why phylogenies based on different genes can yield different branching patterns for the tree of all life.
3. **MAKE CONNECTIONS** Explain how the origin of eukaryotes is thought to have represented a fusion of organisms, leading to extensive horizontal gene transfer. (See Figure 25.10.)

For suggested answers, see Appendix A.

22 Chapter Review



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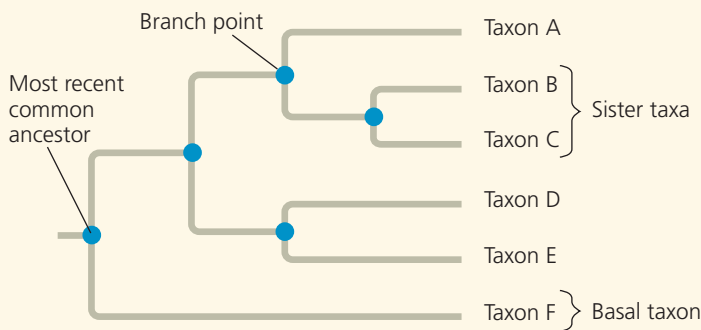
SUMMARY OF KEY CONCEPTS

CONCEPT 22.1

Phylogenies show evolutionary relationships (pp. 520–524)



- Linnaeus's **binomial** classification system gives organisms two-part names: a **genus** plus a specific epithet.
- In the Linnaean system, species are grouped in increasingly broad **taxa**: Related genera are placed in the same **family**, families in **orders**, orders in **classes**, classes in **phyla**, phyla in **kingdoms**, and (more recently) kingdoms in **domains**.
- Systematists depict evolutionary relationships as branching **phylogenetic trees**. Many systematists propose that classification be based entirely on evolutionary relationships.



- Unless branch lengths are proportional to time or genetic change, a phylogenetic tree indicates only patterns of descent.
- Much information can be learned about a species from its evolutionary history; hence, phylogenies are useful in a wide range of applications.

? Humans and chimpanzees are sister species. Explain what this statement means.

CONCEPT 22.2

Phylogenies are inferred from morphological and molecular data (pp. 524–525)

- Organisms with similar morphologies or DNA sequences are likely to be more closely related than organisms with very different structures and genetic sequences.
- To infer phylogeny, **homology** (similarity due to shared ancestry) must be distinguished from **analogy** (similarity due to convergent evolution).
- Computer programs are used to align comparable DNA sequences and to distinguish molecular homologies from coincidental matches between taxa that diverged long ago.

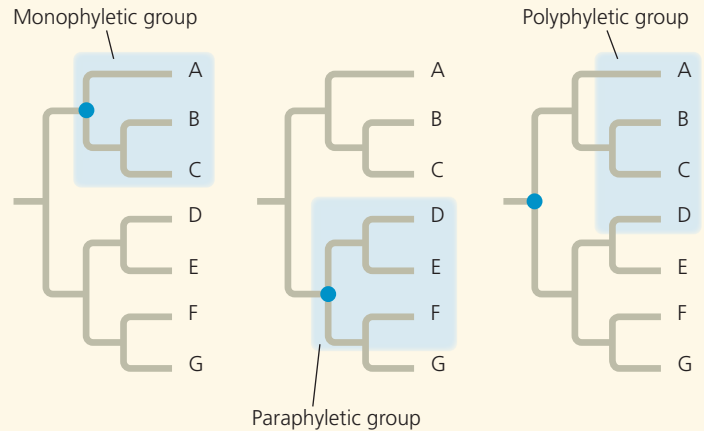
? Why is it necessary to distinguish homology from analogy to infer phylogeny?

CONCEPT 22.3

Shared characters are used to construct phylogenetic trees (pp. 525–531)

- A **clade** is a monophyletic group that includes an ancestral species and all of its descendants.

- Clades can be distinguished by their **shared derived characters**.



- Among phylogenies, the most parsimonious tree is the one that requires the fewest evolutionary changes. The most likely tree is the one based on the most likely pattern of changes.
- Well-supported phylogenetic hypotheses are consistent with a wide range of data.

? Explain the logic of using shared derived characters to infer phylogeny.

CONCEPT 22.4

An organism's evolutionary history is documented in its genome (pp. 531–532)

- Orthologous genes** are homologous genes found in different species as a result of speciation. **Paralogous genes** are homologous genes within a species that result from gene duplication; such genes can diverge and potentially take on new functions.
- Distantly related species often have many orthologous genes. The small variation in gene number in organisms of varying complexity suggests that genes are versatile and may have multiple functions.

? When reconstructing phylogenies, is it more useful to compare orthologous or paralogous genes? Explain.

CONCEPT 22.5

Molecular clocks help track evolutionary time (pp. 532–534)

- Some regions of DNA change at a rate consistent enough to serve as a **molecular clock**, a method of estimating the date of past evolutionary events based on the amount of genetic change. Other DNA regions change in a less predictable way.
- Molecular clock analyses suggest that the most common strain of HIV jumped from primates to humans in the early 1990s.

? Describe some assumptions and limitations of molecular clocks.

CONCEPT 22.6

Our understanding of the tree of life continues to change based on new data (pp. 534–536)

- Past classification systems have given way to the current view of the tree of life, which consists of three great domains: Bacteria, Archaea, and Eukarya.

- Phylogenies based in part on rRNA genes suggest that eukaryotes are most closely related to archaea, while data from some other genes suggest a closer relationship to bacteria.
- Genetic analyses indicate that extensive **horizontal gene transfer** has occurred throughout the evolutionary history of life.

? Why was the five-kingdom system abandoned for a three-domain system?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. **EVOLUTION CONNECTION** Darwin suggested looking at a species' close relatives to learn what its ancestors may have been like. Explain how his suggestion anticipates recent methods, such as phylogenetic bracketing and the use of outgroups in cladistic analysis.



PRACTICE TEST
goo.gl/iAsVgL

9. **SCIENTIFIC INQUIRY • DRAW IT** (a) Draw a phylogenetic tree based on characters 1–5 in the table below. Place hatch marks on the tree to indicate the origin(s) of characters 1–6. (b) Assume that tuna and dolphins are sister species and redraw the phylogenetic tree accordingly. Use hatch marks to indicate the origin(s) of characters 1–6. (c) Determine how many evolutionary changes are required in each tree. Identify the most parsimonious tree.

Character	Lancelet (outgroup)	Lamprey	Tuna	Salamander	Turtle	Leopard	Dolphin
(1) Backbone	0	1	1	1	1	1	1
(2) Hinged jaw	0	0	1	1	1	1	1
(3) Four limbs	0	0	0	1	1	1	1*
(4) Amnion	0	0	0	0	1	1	1
(5) Milk	0	0	0	0	0	1	1
(6) Dorsal fin	0	0	1	0	0	0	1

*Although adult dolphins have only two obvious limbs (their flippers), as embryos they have two hind-limb buds, for a total of four limbs.

10. **WRITE ABOUT A THEME: INFORMATION** In a short essay (100–150 words), explain how genetic information—along with an understanding of the process of descent with modification—enables scientists to reconstruct phylogenies that extend hundreds of millions of years back in time.

11. SYNTHESIZE YOUR KNOWLEDGE



This West Indian manatee (*Trichechus manatus*) is an aquatic mammal. Like amphibians and reptiles, mammals are tetrapods (vertebrates with four limbs). Explain why manatees are considered tetrapods even though they lack hind limbs, and suggest traits that manatees likely share with leopards and other mammals (see Figure 22.12b). Discuss how early members of the manatee lineage might have differed from today's manatees.

For selected answers, see Appendix A.



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▲ **Figure 23.1** Is this finch evolving?

KEY CONCEPTS

- 23.1** Genetic variation makes evolution possible
- 23.2** The Hardy-Weinberg equation can be used to test whether a population is evolving
- 23.3** Natural selection, genetic drift, and gene flow can alter allele frequencies in a population
- 23.4** Natural selection is the only mechanism that consistently causes adaptive evolution

The Smallest Unit of Evolution

One common misconception about evolution is that individual organisms evolve. It is true that natural selection acts on individuals: Each organism's traits affect its survival and reproductive success compared with those of other individuals. But the evolutionary impact of natural selection is only apparent in how a *population* of organisms changes over time.

Consider the medium ground finch (*Geospiza fortis*), a seed-eating bird that inhabits the Galápagos Islands (**Figure 23.1**). In 1977, the *G. fortis* population on the island of Daphne Major was decimated by a long period of drought: Of some 1,200 birds, only 180 survived. Researchers Peter and Rosemary Grant observed that during the drought, small, soft seeds were in short supply. The finches mostly fed on large, hard seeds that were more plentiful. Birds with larger, deeper beaks were better able to crack and eat these larger seeds, and they survived at a higher rate than did finches with smaller beaks. Since beak depth is an inherited trait in these birds, the offspring of surviving birds also tended to have deep beaks. As a result, the average beak depth in the next generation of *G. fortis* was greater than it had been in the pre-drought population (**Figure 23.2**). The finch population had evolved by natural selection. However, the *individual* finches did not evolve. Each bird had a beak of a particular size, which did not grow larger during the drought. Rather, the proportion of large



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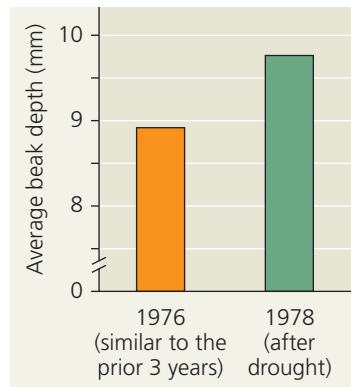
Get Ready for This Chapter

► **Figure 23.2 Evidence of selection by food source.**

The data represent adult beak depth measurements of medium ground finches hatched in the generations before and after the 1977 drought. In one generation, natural selection resulted in a larger average beak size in the population.



Instructors: A related Experimental Inquiry Tutorial can be assigned in MasteringBiology.



beaks in the population increased from generation to generation: The population evolved, not its individual members.

Focusing on evolutionary change in populations, we can define evolution on its smallest scale, called **microevolution**, as a change in allele frequencies in a population over generations. As you will see in this chapter, natural selection is not the only cause of microevolution. In fact, there are three main mechanisms that can cause allele frequency change: natural selection, genetic drift (chance events that alter allele frequencies), and gene flow (the transfer of alleles between populations). Each of these mechanisms has distinctive effects on the genetic composition of populations. However, only natural selection consistently improves the degree to which organisms are well suited for life in their environment (adaptation). Before we examine natural selection and adaptation more closely, let's revisit a prerequisite for these processes in a population: genetic variation.

CONCEPT 23.1

Genetic variation makes evolution possible

In *The Origin of Species*, Darwin provided abundant evidence that life on Earth has evolved over time, and he proposed natural selection as the primary mechanism for that change. He observed that individuals differ in their inherited traits and that selection acts on such differences, leading to evolutionary change. Although Darwin realized that variation in heritable traits is a prerequisite for evolution, he did not know precisely how organisms pass heritable traits to their offspring.

Just a few years after Darwin published *The Origin of Species*, Gregor Mendel wrote a groundbreaking paper on inheritance in pea plants (see Concept 14.1). In that paper, Mendel proposed a model of inheritance in which organisms transmit discrete heritable units (now called genes) to their offspring. Although Darwin did not know about genes, Mendel's paper set the stage for understanding the genetic differences on



▲ **Figure 23.3 Phenotypic variation in horses.** In horses, coat color varies along a continuum and is influenced by multiple genes.

which evolution is based. Here we'll examine such genetic differences and how they are produced.

Genetic Variation

Individuals within all species vary in their phenotypic traits. Among humans, for example, you can easily observe phenotypic variation in facial features, height, and voice. Indeed, individual variation occurs in all species. And though you cannot identify a person's blood group (A, B, AB, or O) from his or her appearance, this and many other molecular traits also vary extensively among individuals.

Such phenotypic variations often reflect **genetic variation**, differences among individuals in the composition of their genes or other DNA sequences. Some heritable phenotypic differences occur on an "either-or" basis, such as the flower colors of Mendel's pea plants: Each plant had flowers that were either purple or white (see Figure 14.3). Characters that vary in this way are typically determined by a single gene locus, with different alleles producing distinct phenotypes. In contrast, other phenotypic differences vary in gradations along a continuum. Such variation usually results from the influence of two or more genes on a single phenotypic character. In fact, many phenotypic characters are influenced by multiple genes, including coat color in horses (**Figure 23.3**), seed number in maize (corn), and height in humans.

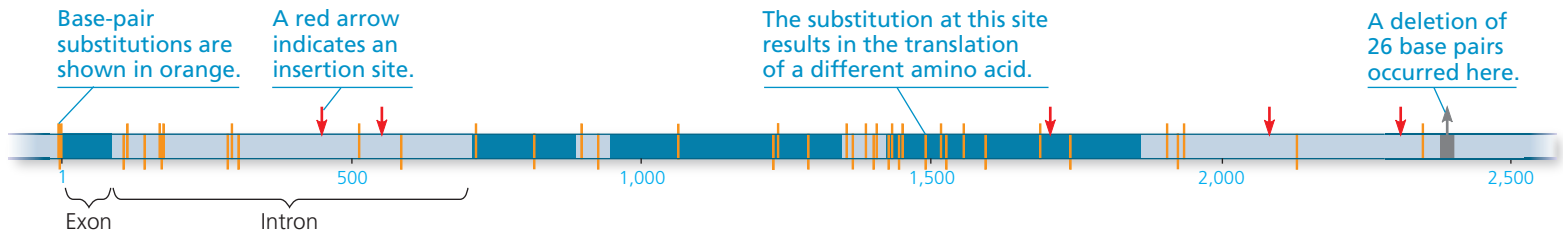
How much do genes and other DNA sequences vary from one individual to another? Genetic variation at the whole-gene level (*gene variability*) can be quantified as the average percentage of loci that are heterozygous. (Recall that a heterozygous individual has two different alleles for a given locus, whereas a homozygous individual has two identical alleles for that locus.) As an example, on average the fruit fly *Drosophila melanogaster* is heterozygous for about 1,920 of its 13,700 loci (14%) and homozygous for all the rest.

Considerable genetic variation can also be measured at the molecular level of DNA (*nucleotide variability*). But little of this variation results in phenotypic variation. Why? Many nucleotide variations occur within *introns*, noncoding

▼ **Figure 23.4 Extensive genetic variation at the molecular level.** This diagram summarizes data from a study comparing the DNA sequence of the alcohol dehydrogenase (*Adh*) gene in several fruit flies (*Drosophila melanogaster*). The *Adh* gene has

four exons (dark blue) separated by introns (light blue); the exons include the coding regions that are ultimately translated into the amino acids of the *Adh* enzyme (see Figure 5.1). Only one substitution has a phenotypic effect, producing a different form of the *Adh* enzyme.

MAKE CONNECTIONS ▶ Review Figures 17.6 and 17.11. Explain how a base-pair substitution that alters a coding region of the *Adh* locus could have no effect on amino acid sequence. Then explain how an insertion in an exon could have no effect on the protein produced.



segments of DNA lying between *exons*, the regions retained in mRNA after RNA processing (see Figure 17.12). And of the variations that occur within exons, most do not cause a change in the amino acid sequence of the protein encoded by the gene. For example, in the sequence comparison shown in **Figure 23.4**, there are 43 nucleotide sites with variable base pairs (where substitutions have occurred), as well as several sites where insertions or deletions have occurred. Although 18 variable sites occur within the four exons of the *Adh* gene, only one of these variations (at site 1,490) results in an amino acid change. Note, however, that this single variable site is enough to cause genetic variation at the level of the gene—and hence two different forms of the *Adh* enzyme are produced.

It is important to bear in mind that some phenotypic variation does not result from genetic differences among individuals (**Figure 23.5** shows a striking example in a caterpillar of the southwestern United States). Phenotype is the product of an inherited genotype and many environmental influences (see Concept 14.3). In a human example, body-builders alter their phenotypes dramatically but do not pass their huge muscles on to the next generation. In general, only the genetically determined part of phenotypic variation can have evolutionary consequences. As such, genetic variation provides the raw material for evolutionary change: Without genetic variation, evolution cannot occur.

▼ **Figure 23.5 Nonheritable variation.** These caterpillars of the moth *Nemoria arizonaria* owe their different appearances to chemicals in their diets, not to differences in their genotypes. (a) Caterpillars raised on a diet of oak flowers resemble the flowers, whereas (b) their siblings raised on oak leaves resemble oak twigs.



Sources of Genetic Variation

The genetic variation on which evolution depends originates when mutation, gene duplication, or other processes produce new alleles and new genes. Genetic variants can be produced rapidly in organisms with short generation times. Sexual reproduction can also result in genetic variation as existing genes are arranged in new ways.

Formation of New Alleles

New alleles can arise by *mutation*, a change in the nucleotide sequence of an organism's DNA. A change of as little as one base in a gene—a “point mutation”—can have a significant impact on phenotype, as in sickle-cell disease (see Figure 17.26). We might expect that this would be the case: Organisms reflect many generations of past selection, and hence their phenotypes tend to be suited for life in their environments. As a result, most new mutations that alter a phenotype are at least slightly harmful.

In some cases, natural selection quickly removes such harmful alleles. In diploid organisms, however, harmful alleles that are recessive can be hidden from selection. Indeed, a harmful recessive allele can persist for generations by propagation in heterozygous individuals (where its harmful effects can be masked by the more favorable dominant allele). Such “heterozygote protection” maintains a huge pool of alleles that might not be favored under present conditions, but that could be beneficial if the environment changes.

While many mutations are harmful, many others are not. Recall that much of the DNA in eukaryotic genomes does not encode proteins (see Figure 20.6). Point mutations in these noncoding regions generally result in **neutral variation**, differences in DNA sequence that do not confer a selective advantage or disadvantage. The redundancy in the genetic code is another source of neutral variation: Even a point mutation in a gene that encodes a protein

will have no effect on the protein's function if the amino acid composition is not changed. And even where there is a change in the amino acid, it may not affect the protein's shape and function. Moreover, as you will see later in this chapter, a mutant allele may on rare occasions actually make its bearer better suited to the environment, enhancing reproductive success.

Finally, note that in multicellular organisms, only mutations in cell lines that produce gametes can be passed to offspring. In plants and fungi, this is not as limiting as it may sound, since many different cell lines can produce gametes. But in most animals, the majority of mutations occur in somatic cells and are not passed to offspring.

Altering Gene Number or Position

Chromosomal changes that delete, disrupt, or rearrange many loci are usually harmful. However, when such large-scale changes leave genes intact, they may not affect the organisms' phenotype. In rare cases, chromosomal rearrangements may even be beneficial. For example, the translocation of part of one chromosome to a different chromosome could link genes in a way that produces a positive effect.

A key potential source of variation is the duplication of genes due to errors in meiosis (such as unequal crossing over), slippage during DNA replication, or the activities of transposable elements (see Concept 20.5). Duplications of large chromosome segments, like other chromosomal aberrations, are often harmful, but the duplication of smaller pieces of DNA may not be. Gene duplications that do not have severe effects can persist over generations, allowing mutations to accumulate. The result is an expanded genome with new genes that may take on new functions.

Such increases in gene number appear to have played a major role in evolution. For example, the remote ancestors of mammals had a single gene for detecting odors that has since been duplicated many times. As a result, humans today have about 380 functional olfactory receptor genes, and mice have about 1,200. This dramatic proliferation of olfactory genes probably helped early mammals, enabling them to detect faint odors and to distinguish among many different smells.

Rapid Reproduction

Mutation rates tend to be low in plants and animals, averaging about one mutation in every 100,000 genes per generation, and they are often even lower in prokaryotes. But prokaryotes have many more generations per unit of time, so mutations can quickly generate genetic variation in their populations. The same is true of viruses. For instance, HIV has a generation time of about two days (that is, it takes two days for a newly formed virus to produce the next generation of viruses). HIV also has an RNA genome, which has a much higher mutation rate than a typical DNA genome because of the lack of RNA repair mechanisms in host cells (see Concept 26.2). For this reason, single-drug treatments are unlikely to be effective against HIV:

Mutant forms of the virus that are resistant to a particular drug would tend to proliferate in relatively short order. The most effective AIDS treatments to date have been drug "cocktails" that combine several medications. This approach has worked well because it is less likely that a set of mutations that together confer resistance to *all* the drugs will occur in a short time period.

Sexual Reproduction

In organisms that reproduce sexually, most of the genetic variation in a population results from the unique combination of alleles that each individual receives from its parents. Of course, at the nucleotide level, all the differences among these alleles have originated from past mutations. Sexual reproduction then shuffles existing alleles and deals them at random to produce individual genotypes.

Three mechanisms contribute to this shuffling: crossing over, independent assortment of chromosomes, and fertilization (see Concept 13.4). During meiosis, homologous chromosomes, one inherited from each parent, trade some of their alleles by crossing over. These homologous chromosomes and the alleles they carry are then distributed at random into gametes. Then, because myriad possible mating combinations exist in a population, fertilization typically brings together gametes that have different genetic backgrounds. The combined effects of these three mechanisms ensure that sexual reproduction rearranges existing alleles into fresh combinations each generation, providing much of the genetic variation that makes evolution possible.



Animation: Origins of Genetic Variation

CONCEPT CHECK 23.1

1. Explain why genetic variation within a population is a prerequisite for evolution.
2. Of all the mutations that occur in a population, why do only a small fraction become widespread?
3. **MAKE CONNECTIONS** > If a population stopped reproducing sexually (but still reproduced asexually), how would its genetic variation be affected over time? Explain. (See Concept 13.4.)

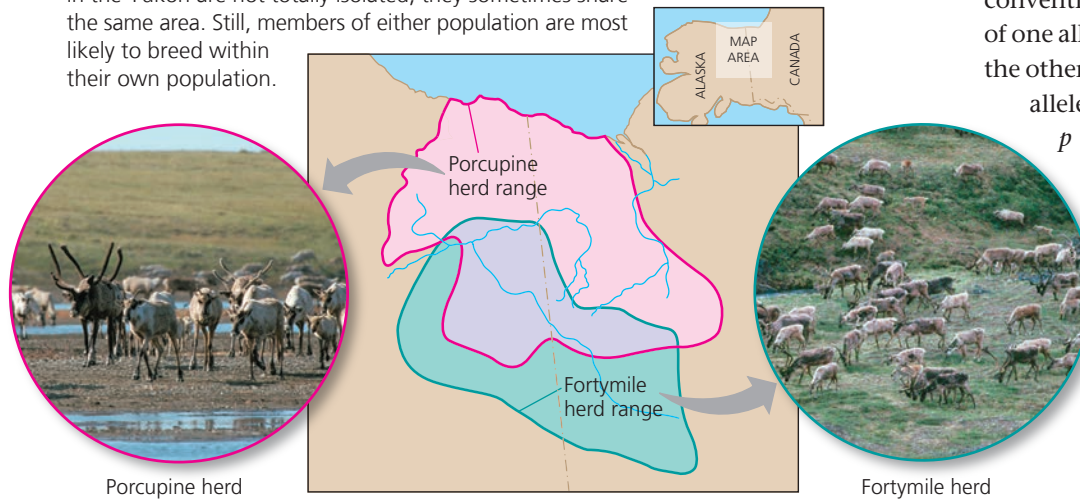
For suggested answers, see Appendix A.

CONCEPT 23.2

The Hardy-Weinberg equation can be used to test whether a population is evolving

Although the individuals in a population must differ genetically for evolution to occur, the presence of genetic variation does not guarantee that a population will evolve. For that to happen, one or more factors that cause evolution must be at work. In this section, we'll explore one way to test whether evolution is occurring in a population. First, let's clarify what we mean by a population.

▼ **Figure 23.6 One species, two populations.** These two caribou populations in the Yukon are not totally isolated; they sometimes share the same area. Still, members of either population are most likely to breed within their own population.



When studying a locus with two alleles, the convention is to use p to represent the frequency of one allele and q to represent the frequency of the other allele. Thus, p , the frequency of the C^R allele in the gene pool of this population, is $p = 0.8$ (80%). And because there are only two alleles for this gene, the frequency of the C^W allele, represented by q , must be $q = 1 - p = 0.2$ (20%). For loci that have more than two alleles, the sum of all allele frequencies must still equal 1 (100%).

Next we'll see how allele and genotype frequencies can be used to test whether evolution is occurring in a population.

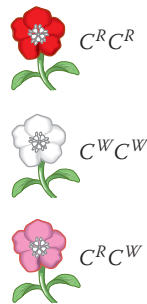
Gene Pools and Allele Frequencies

A **population** is a group of individuals of the same species that live in the same area and interbreed, producing fertile offspring. Different populations of a species may be isolated geographically from one another, exchanging genetic material only rarely. Such isolation is common for species that live on widely separated islands or in different lakes. But not all populations are isolated (Figure 23.6). Still, members of a population typically breed with one another and thus on average are more closely related to each other than to members of other populations.

We can characterize a population's genetic makeup by describing its **gene pool**, which consists of all copies of every type of allele at every locus in all members of the population. If only one allele exists for a particular locus in a population, that allele is said to be *fixed* in the gene pool, and all individuals are homozygous for that allele. But if there are two or more alleles for a particular locus in a population, individuals may be either homozygous or heterozygous.

For example, imagine a population of 500 wildflower plants with two alleles, C^R and C^W , for a locus that codes for flower pigment. These alleles show incomplete dominance; thus, each genotype has a distinct phenotype. Plants homozygous for the C^R allele ($C^R C^R$) produce red pigment and have red flowers; plants homozygous for the C^W allele ($C^W C^W$) produce no red pigment and have white flowers; and heterozygotes ($C^R C^W$) produce some red pigment and have pink flowers.

Each allele has a frequency (proportion) in the population. For example, suppose our population has 320 plants with red flowers, 160 with pink flowers, and 20 with white flowers. Because these are diploid organisms, these 500 individuals have a total of 1,000 copies of the gene for flower color. The C^R allele accounts for 800 of these copies ($320 \times 2 = 640$ for $C^R C^R$ plants, plus $160 \times 1 = 160$ for $C^R C^W$ plants). Thus, the frequency of the C^R allele is $800/1,000 = 0.8$ (80%).



The Hardy-Weinberg Equation

One way to assess whether natural selection or other factors are causing evolution at a particular locus is to determine what the genetic makeup of a population would be if it were *not* evolving at that locus. We can then compare that scenario with the data we actually observed for the population. If there are no differences, we can conclude that the population is not evolving. If there are differences, this suggests that the population may be evolving—and then we can try to figure out why.

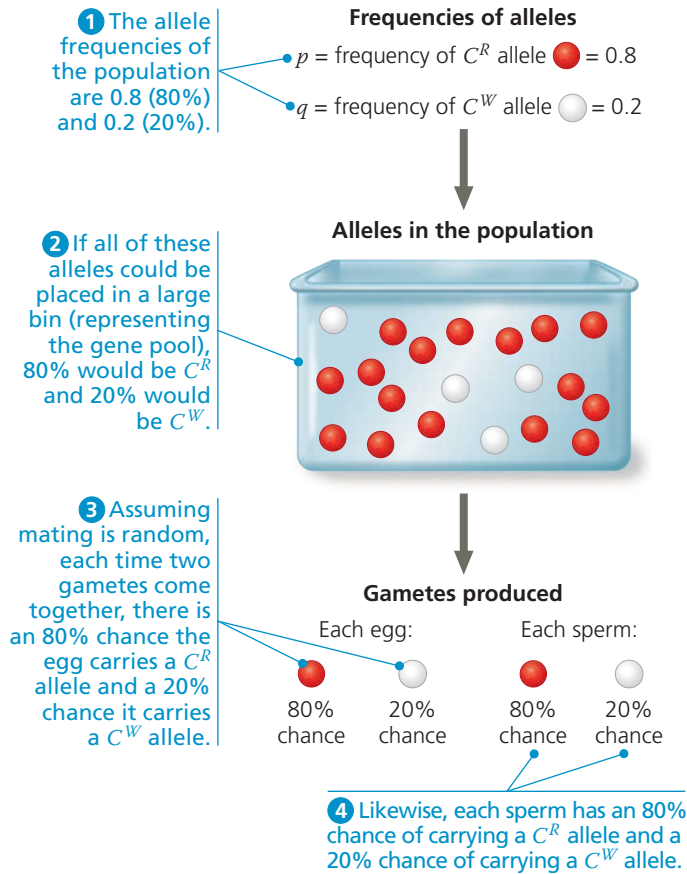
Hardy-Weinberg Equilibrium

In a population that is not evolving, allele and genotype frequencies will remain constant from generation to generation, provided that only Mendelian segregation and recombination of alleles are at work. Such a population is said to be in **Hardy-Weinberg equilibrium**, named for the British mathematician and German physician, respectively, who independently developed this idea in 1908.

To determine whether a population is in Hardy-Weinberg equilibrium, it is helpful to think about genetic crosses in a new way. Previously, we used Punnett squares to determine the genotypes of offspring in a genetic cross (see Figure 14.5). Here, instead of considering the possible allele combinations from one cross, we'll consider the combination of alleles in *all* of the crosses in a population.

Imagine that all the alleles for a given locus from all the individuals in a population are placed in a large bin (Figure 23.7). We can think of this bin as holding the population's gene pool for that locus. "Reproduction" occurs by selecting alleles at random from the bin; somewhat similar events occur in nature when fish release sperm and eggs into the water or when pollen (containing plant sperm) is blown about by the wind. By viewing reproduction as a process of randomly selecting and combining alleles from the bin (the gene pool), we are in effect assuming that mating occurs at random—that is, that all male-female matings are equally likely.

▼ **Figure 23.7** Selecting alleles at random from a gene pool.



DRAW IT ► Draw a similar bin that contains six white balls instead of four. For the frequency of C^R in the bin to remain equal to 0.8, how many red balls should the bin contain?

BioFlix® Animation: Allele Frequencies

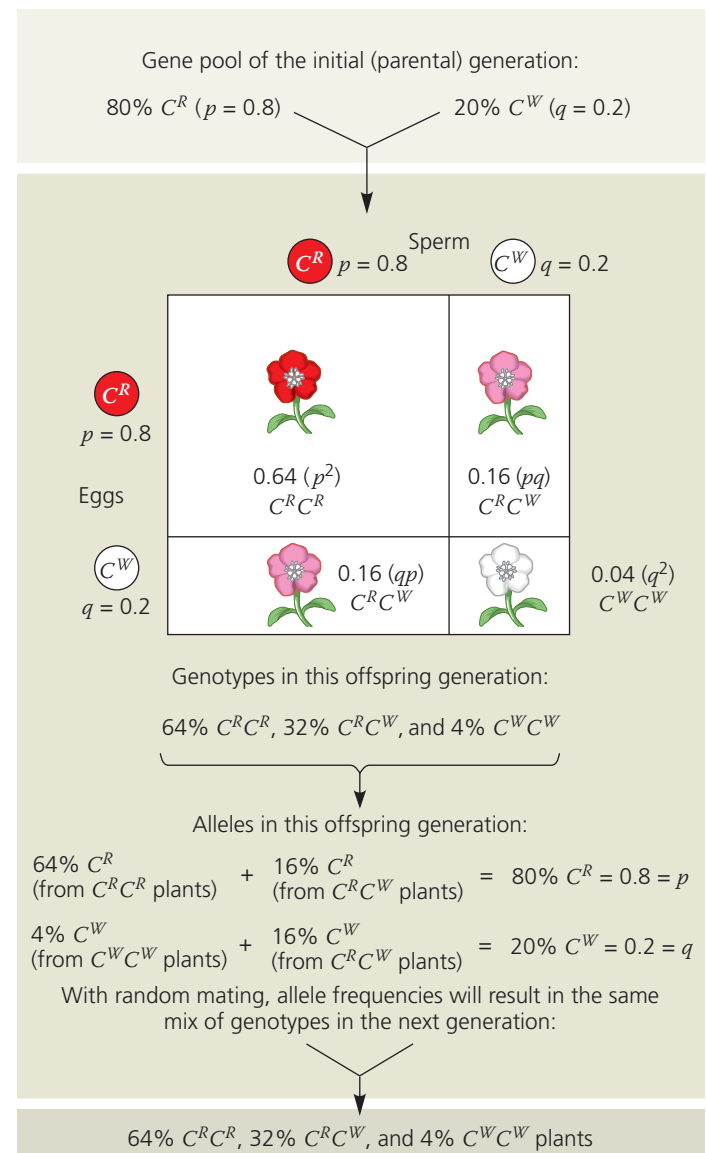
Let's apply the bin analogy to the hypothetical wildflower population discussed earlier. In that population of 500 flowers, the frequency of the allele for red flowers (C^R) is $p = 0.8$, and the frequency of the allele for white flowers (C^W) is $q = 0.2$. This implies that a bin holding all 1,000 copies of the flower-color gene in the population would contain 800 C^R alleles and 200 C^W alleles. Assuming that gametes are formed by selecting alleles at random from the bin, the probability that an egg or sperm contains a C^R or C^W allele is equal to the frequency of these alleles in the bin. Thus, as shown in Figure 23.7, each egg has an 80% chance of containing a C^R allele and a 20% chance of containing a C^W allele; the same is true for each sperm.

Using the rule of multiplication (see Figure 14.9), we can now calculate the frequencies of the three possible genotypes, assuming random unions of sperm and eggs. The probability that two C^R alleles will come together is $p \times p = p^2 = 0.8 \times 0.8 = 0.64$. Thus, about 64% of the plants in the next generation will have the genotype $C^R C^R$. The frequency of $C^W C^W$ individuals is expected to be about $q \times q = q^2 = 0.2 \times 0.2 = 0.04$, or 4%. $C^R C^W$ heterozygotes can arise in

two different ways. If the sperm provides the C^R allele and the egg provides the C^W allele, the resulting heterozygotes will be $p \times q = 0.8 \times 0.2 = 0.16$, or 16% of the total. If the sperm provides the C^W allele and the egg the C^R allele, the heterozygous offspring will make up $q \times p = 0.2 \times 0.8 = 0.16$, or 16%. The frequency of heterozygotes is thus the sum of these possibilities: $pq + qp = 2pq = 0.16 + 0.16 = 0.32$, or 32%.

As shown in Figure 23.8, the genotype frequencies in the next generation must add up to 1 (100%). Thus, the equation for Hardy-Weinberg equilibrium states that at a locus with

▼ **Figure 23.8** Hardy-Weinberg equilibrium. In our wildflower population, the gene pool remains constant from one generation to the next. Mendelian processes alone do not alter frequencies of alleles or genotypes.



WHAT IF? ► If the frequency of the C^R allele were 0.6, predict the frequencies of the $C^R C^R$, $C^R C^W$, and $C^W C^W$ genotypes.

Figure Walkthrough

two alleles, the three genotypes will appear in the following proportions:

$$p^2 + 2pq + q^2 = 1$$

Expected frequency of genotype C^RC^R

Expected frequency of genotype C^RC^W

Expected frequency of genotype C^WC^W

Note that for a locus with two alleles, only three genotypes are possible (in this case, C^RC^R , C^RC^W , and C^WC^W). As a result, the sum of the frequencies of the three genotypes must equal 1 (100%) in *any* population—regardless of whether the population is in Hardy-Weinberg equilibrium. The key point is that a population is in Hardy-Weinberg equilibrium only if the observed genotype frequency of one homozygote is p^2 , the observed frequency of the other homozygote is q^2 , and the observed frequency of heterozygotes is $2pq$. Finally, as suggested by Figure 23.8, if a population such as our wildflowers is in Hardy-Weinberg equilibrium and its members continue to mate randomly generation after generation, allele and genotype frequencies will remain constant. The system operates somewhat like a deck of cards: No matter how many times the deck is reshuffled to deal out new hands, the deck itself remains the same. Aces do not grow more numerous than jacks. And the repeated shuffling of a population’s gene pool over the generations cannot, in itself, change the frequency of one allele relative to another.

Conditions for Hardy-Weinberg Equilibrium

The Hardy-Weinberg approach describes a population that is not evolving. This can occur if a population meets all five of the conditions for Hardy-Weinberg equilibrium listed in Table 23.1. But in nature, the allele and genotype frequencies of a population often *do* change over time. Such changes can occur when at least one of the conditions for Hardy-Weinberg equilibrium is not met.

Although departure from the conditions in Table 23.1 is common—resulting in evolutionary change—it is also common for natural populations to be in Hardy-Weinberg equilibrium for specific genes. One way this can happen is if selection alters allele frequencies at some loci but not others. In addition, some populations evolve so slowly that the changes in their allele and genotype frequencies are difficult to distinguish from those predicted for a non-evolving population.

Applying the Hardy-Weinberg Equation

The Hardy-Weinberg equation is often used as an initial test of whether evolution is occurring in a population. The equation also has medical applications, such as estimating the percentage of a population carrying the allele for an inherited disease. For example, consider phenylketonuria (PKU), a metabolic disorder that results from homozygosity for a recessive allele. This disorder occurs in about one out of every

Table 23.1 Conditions for Hardy-Weinberg Equilibrium	
Condition	Consequence if Condition Does Not Hold
1. No mutations	The gene pool is modified if mutations occur or if entire genes are deleted or duplicated.
2. Random mating	If individuals mate within a subset of the population, such as near neighbors or close relatives (inbreeding), random mixing of gametes does not occur and genotype frequencies change.
3. No natural selection	Allele frequencies change when individuals with different genotypes show consistent differences in their survival or reproductive success.
4. Extremely large population size	In small populations, allele frequencies fluctuate by chance over time (a process called genetic drift).
5. No gene flow	By moving alleles into or out of populations, gene flow can alter allele frequencies.

Animation: Causes of Evolutionary Change

10,000 babies born in the United States. Left untreated, PKU results in mental disability and other problems. (As described in Concept 14.4, newborns are now routinely tested for PKU, and symptoms can be largely avoided with a diet very low in phenylalanine.)

To apply the Hardy-Weinberg equation, we must assume that no new PKU mutations are being introduced into the population (condition 1) and that people neither choose their mates on the basis of whether or not they carry this gene nor generally mate with close relatives (condition 2). We must also ignore any effects of differential survival and reproductive success among PKU genotypes (condition 3) and assume that there are no effects of genetic drift (condition 4) or of gene flow from other populations into the United States (condition 5). These assumptions are reasonable: The mutation rate for the PKU gene is low, inbreeding and other forms of nonrandom mating are not common in the United States, selection occurs only against the rare homozygotes (and then only if dietary restrictions are not followed), the U.S. population is very large, and populations outside the country have PKU allele frequencies similar to those seen in the United States.

If all these assumptions hold, then the frequency of individuals in the population born with PKU will correspond to q^2 in the Hardy-Weinberg equation (q^2 = frequency of homozygotes). Because the allele is recessive, we must estimate the number of heterozygotes rather than counting them directly as we did with the pink flowers. Recall that there is one PKU occurrence per 10,000 births, which indicates that q^2 = 0.0001. Thus, the frequency (q) of the recessive allele for PKU is

$$q = \sqrt{0.0001} = 0.01$$

and the frequency of the dominant allele is

$$p = 1 - q = 1 - 0.01 = 0.99$$

The frequency of carriers, heterozygous people who do not have PKU but may pass the PKU allele to offspring, is

$$2pq = 2 \times 0.99 \times 0.01 = 0.0198$$

(approximately 2% of the U.S. population)

Remember, the assumption of Hardy-Weinberg equilibrium yields an approximation; the real number of carriers may differ. Still, our calculations suggest that harmful recessive alleles at this and other loci can be concealed in a population because they are carried by healthy heterozygotes. The **Scientific Skills Exercise** provides another opportunity for you to apply the Hardy-Weinberg equation to allele data.

CONCEPT CHECK 23.2

1. A population has 700 individuals, 85 of genotype AA , 320 of genotype Aa , and 295 of genotype aa . What are the frequencies of alleles A and a ?
2. The frequency of allele a is 0.45 for a population in Hardy-Weinberg equilibrium. What are the expected frequencies of genotypes AA , Aa , and aa ?
3. **WHAT IF? >** A locus that affects susceptibility to a degenerative brain disease has two alleles, V and v . In a population, 16 people have genotype VV , 92 have genotype Vv , and 12 have genotype vv . Is this population evolving? Explain.

For suggested answers, see Appendix A.

CONCEPT 23.3

Natural selection, genetic drift, and gene flow can alter allele frequencies in a population

Note again the five conditions required for a population to be in Hardy-Weinberg equilibrium (see Table 23.1). A deviation from any of these conditions is a potential cause of evolution. New mutations (violation of condition 1) can alter allele frequencies, but because mutations are rare, the change from one generation to the next is likely to be very small. Nonrandom mating (violation of condition 2) can affect the frequencies of homozygous and heterozygous genotypes but by itself has no effect on allele frequencies in the gene pool. (Allele frequencies can change if individuals with certain inherited traits are more likely than other individuals to obtain mates. However, such a situation not only causes a deviation from random mating, but also violates condition 3, no natural selection.)

For the rest of this section we will focus on the three mechanisms that alter allele frequencies directly and cause most evolutionary change: natural selection, genetic drift, and gene flow (violations of conditions 3–5).

SCIENTIFIC SKILLS EXERCISE

Using the Hardy-Weinberg Equation to Interpret Data and Make Predictions

Is Evolution Occurring in a Soybean Population? One way to test whether evolution is occurring in a population is to compare the observed genotype frequencies at a locus with those expected for a non-evolving population based on the Hardy-Weinberg equation. In this exercise, you'll test whether a soybean population is evolving at a locus with two alleles, C^G and C^Y , that affect chlorophyll production and hence leaf color.

How the Experiment Was Done Students planted soybean seeds and then counted the number of seedlings of each genotype at day 7 and again at day 21. Seedlings of each genotype could be distinguished visually because the C^G and C^Y alleles show incomplete dominance: $C^G C^G$ seedlings have green leaves, $C^G C^Y$ seedlings have green-yellow leaves, and $C^Y C^Y$ seedlings have yellow leaves.

Data from the Experiment

Time (days)	Number of Seedlings			Total
	Green ($C^G C^G$)	Green-yellow ($C^G C^Y$)	Yellow ($C^Y C^Y$)	
7	49	111	56	216
21	47	106	20	173

INTERPRET THE DATA

1. Use the observed genotype frequencies from the day 7 data to calculate the frequencies of the C^G allele (p) and the C^Y allele (q).

2. Next, use the Hardy-Weinberg equation ($p^2 + 2pq + q^2 = 1$) to calculate the day 7 expected frequencies of genotypes $C^G C^G$, $C^G C^Y$, and $C^Y C^Y$ for a population in Hardy-Weinberg equilibrium.
3. Calculate the observed frequencies of genotypes $C^G C^G$, $C^G C^Y$, and $C^Y C^Y$ at day 7. Compare these frequencies to the expected frequencies calculated in question 2. Is the seedling population in Hardy-Weinberg equilibrium at day 7, or is evolution occurring? Explain your reasoning and identify which genotypes, if any, appear to be selected for or against.
4. Calculate the observed frequencies of genotypes $C^G C^G$, $C^G C^Y$, and $C^Y C^Y$ at day 21. Compare these frequencies to the expected frequencies calculated in question 2 and to the observed frequencies at day 7. Is the seedling population in Hardy-Weinberg equilibrium at day 21, or is evolution occurring? Explain your reasoning and identify which genotypes, if any, appear to be selected for or against.
5. Homozygous $C^Y C^Y$ individuals cannot produce chlorophyll. The ability to photosynthesize becomes more critical as seedlings age and begin to exhaust the supply of food that was stored in the seed from which they emerged. Develop a hypothesis that explains the data for days 7 and 21. Based on this hypothesis, predict how the frequencies of the C^G and C^Y alleles will change beyond day 21.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

The Founder Effect

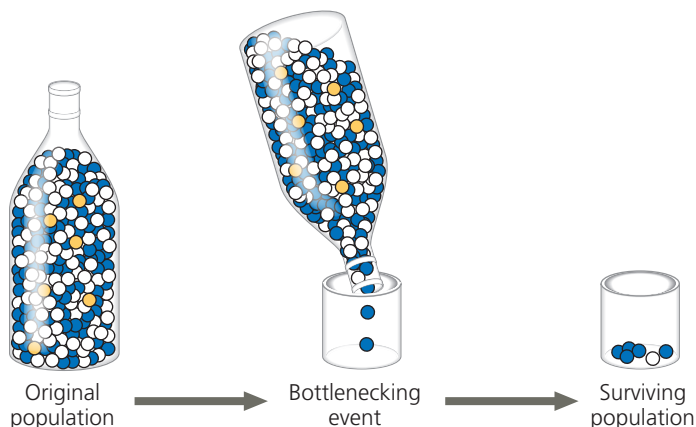
When a few individuals become isolated from a larger population, this smaller group may establish a new population whose gene pool differs from the source population; this is called the **founder effect**. The founder effect might occur, for example, when a few members of a population are blown by a storm to a new island. Genetic drift, in which chance events alter allele frequencies, can occur in such a case because the storm indiscriminately transports some individuals (and their alleles), but not others, from the source population.

The founder effect probably accounts for the relatively high frequency of certain inherited disorders among isolated human populations. For example, in 1814, 15 British colonists founded a settlement on Tristan da Cunha, a group of small islands in the Atlantic Ocean midway between Africa and South America. Apparently, one of the colonists carried a recessive allele for retinitis pigmentosa, a progressive form of blindness that afflicts homozygous individuals. Of the founding colonists' 240 descendants on the island in the late 1960s, four had retinitis pigmentosa. The frequency of the allele that causes this disease is ten times higher on Tristan da Cunha than in the populations from which the founders came.

The Bottleneck Effect

A sudden change in the environment, such as a fire or flood, may drastically reduce the size of a population. A severe drop in population size can cause the **bottleneck effect**, so named because the population has passed through a “bottleneck” that reduces its size (Figure 23.10). By chance alone, certain alleles may be overrepresented among the survivors, others may be underrepresented, and some may be absent altogether. Ongoing genetic drift is likely to have substantial effects on the gene pool until the population becomes large enough that chance events have less impact. But even if a population that has passed through a bottleneck ultimately

▼ **Figure 23.10 The bottleneck effect.** Shaking just a few marbles through the narrow neck of a bottle is analogous to a drastic reduction in the size of a population. By chance, blue marbles are overrepresented in the surviving population and gold marbles are absent.



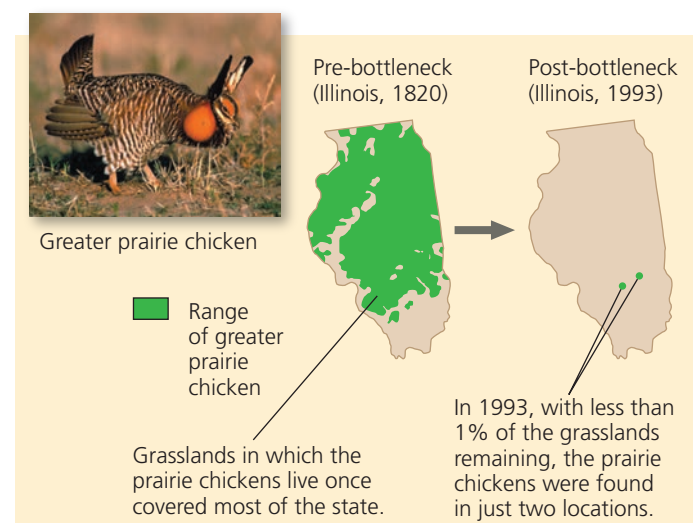
recovers in size, it may have low levels of genetic variation for a long period of time—a legacy of the genetic drift that occurred when the population was small.

Human actions sometimes create severe bottlenecks for other species, as the following example shows.

Case Study: Impact of Genetic Drift on the Greater Prairie Chicken

Millions of greater prairie chickens (*Tympanuchus cupido*) once lived on the prairies of Illinois. As these prairies were converted to farmland and other uses during the 19th and 20th centuries, the number of greater prairie chickens plummeted (Figure 23.11a). By 1993 fewer than 50 birds remained. These few surviving birds had low levels of genetic variation, and less than 50% of their eggs hatched, compared with much higher hatching rates of the larger populations in Kansas and Nebraska (Figure 23.11b).

▼ **Figure 23.11 Genetic drift and loss of genetic variation.**



(a) The Illinois population of greater prairie chickens dropped from millions of birds in the 1800s to fewer than 50 birds in 1993.

Location	Population size	Number of alleles per locus	Percentage of eggs hatched
Illinois			
1930–1960s	1,000–25,000	5.2	93
1993	<50	3.7	<50
Kansas, 1998 (no bottleneck)	750,000	5.8	99
Nebraska, 1998 (no bottleneck)	75,000–200,000	5.8	96

(b) In the small Illinois population, genetic drift led to decreases in the number of alleles per locus and the percentage of eggs hatched.

These data suggest that genetic drift during the bottleneck may have led to a loss of genetic variation and an increase in the frequency of harmful alleles. To investigate this hypothesis, researchers extracted DNA from 15 museum specimens of Illinois greater prairie chickens. Of the 15 birds, 10 had been collected in the 1930s, when there were 25,000 greater prairie chickens in Illinois, and 5 had been collected in the 1960s, when there were 1,000 greater prairie chickens in Illinois. By studying the DNA of these specimens, the researchers were able to obtain a minimum, baseline estimate of how much genetic variation was present in the Illinois population *before* the population shrank to extremely low numbers. This baseline estimate is a key piece of information that is not usually available in cases of population bottlenecks.

The researchers surveyed six loci and found that the 1993 population had fewer alleles per locus than the pre-bottleneck Illinois or the current Kansas and Nebraska populations (see Figure 23.11b). Thus, as predicted, drift had reduced the genetic variation of the small 1993 population. Drift may also have increased the frequency of harmful alleles, leading to the low egg-hatching rate. To counteract these negative effects, 271 birds from neighboring states were added to the Illinois population over four years. This strategy succeeded: New alleles entered the population, and the egg-hatching rate improved to over 90%. Overall, studies on the Illinois greater prairie chicken illustrate the powerful effects of genetic drift in small populations and provide hope that in at least some populations, these effects can be reversed.

Effects of Genetic Drift: A Summary

The examples we've described highlight four key points:

1. Genetic drift is significant in small populations.

Chance events can cause an allele to be disproportionately over- or underrepresented in the next generation. Although chance events occur in populations of all sizes, they tend to alter allele frequencies substantially only in small populations.

2. Genetic drift can cause allele frequencies to change at random.

Because of genetic drift, an allele may increase in frequency one year, then decrease the next; the change from year to year is not predictable. Thus, unlike natural selection, which in a given environment consistently favors some alleles over others, genetic drift causes allele frequencies to change at random over time.

3. Genetic drift can lead to a loss of genetic variation within populations.

By causing allele frequencies to fluctuate randomly over time, genetic drift can eliminate alleles from a population. Because evolution depends on genetic variation, such losses can influence how effectively a population can adapt to a change in the environment.

4. Genetic drift can cause harmful alleles to become fixed.

Alleles that are neither harmful nor beneficial can

be lost or become fixed (reach a frequency of 100%) by chance through genetic drift. In very small populations, genetic drift can also cause alleles that are slightly harmful to become fixed. When this occurs, the population's survival can be threatened (as in greater prairie chickens).

Gene Flow

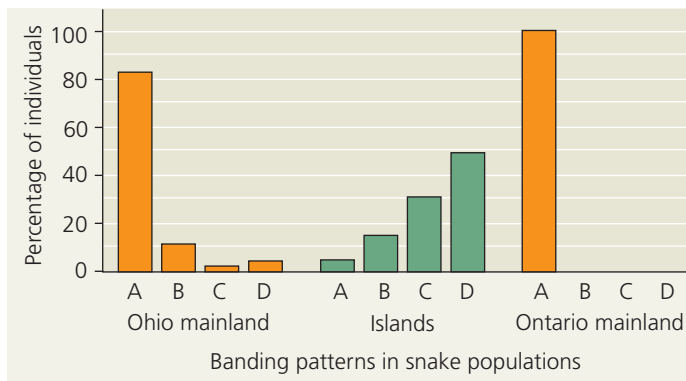
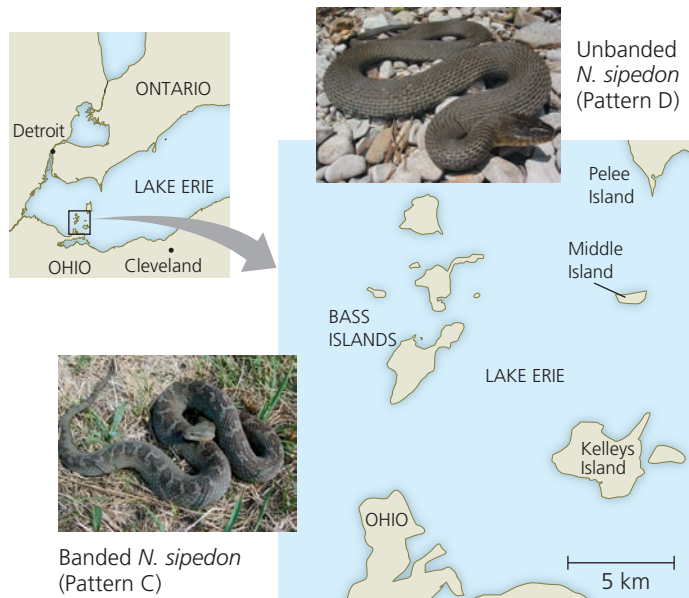
Natural selection and genetic drift are not the only phenomena affecting allele frequencies. Allele frequencies can also change by **gene flow**, the transfer of alleles into or out of a population due to the movement of fertile individuals or their gametes. For example, suppose that near our original hypothetical wildflower population there is another population consisting primarily of white-flowered individuals (C^wC^w). Insects carrying pollen from these plants may fly to and pollinate plants in our original population. The introduced C^w alleles would modify our original population's allele frequencies in the next generation. Because alleles are transferred between populations, gene flow tends to reduce the genetic differences between populations. In fact, if it is extensive enough, gene flow can result in two populations combining into a single population with a common gene pool.

Alleles transferred by gene flow can also affect how well populations are adapted to local environmental conditions. For instance, mainland and island populations of the Lake Erie water snake (*Nerodia sipedon*) differ in their color patterns: Nearly all snakes from the Ohio or Ontario mainlands are strongly banded, whereas the majority of snakes from islands are unbanded or intermediate (**Figure 23.12**). Banding coloration is an inherited trait, determined by a few loci (with alleles that encode bands being dominant to alleles that encode the absence of bands). On islands, water snakes live along rocky shorelines, while on the mainland, they live in marshes. Snakes without bands are more well camouflaged in island habitats than are snakes with bands. Hence, on islands, snakes without bands survive at higher rates than do snakes with bands.

These data indicate that snakes without bands are favored by natural selection in island populations. Thus, we might expect that *all* snakes on islands would lack bands. Why is this not the case? The answer lies in gene flow from the mainland. In any given year, 3 to 10 snakes from the mainland swim to the islands and join the populations there. As a result, each year such migrants transfer alleles for banded coloration from the mainland (where nearly all snakes have bands) to the islands. This ongoing gene flow has prevented selection from removing all of the alleles for banded coloration from island populations—thereby preventing island populations from adapting fully to local conditions.

Gene flow can also transfer alleles that improve the ability of populations to adapt to local conditions. For example, gene flow has resulted in the worldwide spread of several insecticide resistance alleles in the mosquito *Culex pipiens*,

▼ **Figure 23.12 Gene flow and local adaptation in the Lake Erie water snake (*Nerodia sipedon*).** Researchers assigned letters to variations in coloration in *N. sipedon* populations. Color pattern A is strong banding, patterns B and C are intermediate banding, and pattern D is no banding. Banding is advantageous for camouflage in mainland environments, whereas having no bands is advantageous in island environments. However, gene flow from the mainland causes banding to persist in island populations.



WHAT IF? > Suppose a severe weather event caused island populations to decrease in size but did not affect the size of mainland populations. Predict how gene flow from the mainland would affect color patterns in island populations. Explain.

a vector of West Nile virus and other diseases. Each of these alleles has a unique genetic signature that allowed researchers to document that it arose by mutation in only one or a few geographic locations. In their population of origin, these alleles increased because they provided insecticide resistance. These alleles were then transferred to new populations, where again, their frequencies increased as a result of natural selection.

Finally, gene flow has become an increasingly important agent of evolutionary change in human populations. Humans today move much more freely about the world than in the past. As a result, mating is more common between

members of populations that previously had very little contact, leading to an exchange of alleles and fewer genetic differences between those populations.

BioFlix® Animation: Natural Selection, Genetic Drift, and Gene Flow

CONCEPT CHECK 23.3

1. In what sense is natural selection more “predictable” than genetic drift?
2. Distinguish genetic drift from gene flow in terms of (a) how they occur and (b) their implications for future genetic variation in a population.
3. **WHAT IF? >** Suppose two plant populations exchange pollen and seeds. In one population, individuals of genotype *AA* are most common (9,000 *AA*, 900 *Aa*, 100 *aa*), while the opposite is true in the other population (100 *AA*, 900 *Aa*, 9,000 *aa*). If neither allele has a selective advantage, what will happen over time to the allele and genotype frequencies of these populations?

For suggested answers, see Appendix A.

CONCEPT 23.4

Natural selection is the only mechanism that consistently causes adaptive evolution

Evolution by natural selection is a blend of chance and “sorting”: chance in the creation of new genetic variations (as in mutation) and sorting as natural selection favors some alleles over others. Because of this favoring process, the outcome of natural selection is *not* random. Instead, natural selection consistently increases the frequencies of alleles that provide reproductive advantage, thus leading to adaptive evolution.

Natural Selection: A Closer Look

To see how natural selection can cause adaptive evolution, we’ll begin with the concept of relative fitness and the different ways that selection acts on an organism’s phenotype.

Relative Fitness

The phrases “struggle for existence” and “survival of the fittest” are commonly used to describe natural selection, but these expressions are misleading if always taken to mean direct competitive contests among individuals. There *are* animal species in which individuals, usually the males, lock horns or otherwise do combat to determine mating privilege. But reproductive success is generally more subtle and depends on many factors besides outright battle. For example, a barnacle that is more efficient at collecting food than its neighbors may have greater stores of energy and hence be able to produce a larger number of eggs. A moth may have more offspring than other moths in the same population because its body colors more effectively conceal

it from predators, improving its chance of surviving long enough to produce more offspring. These examples illustrate how in a given environment, certain traits can lead to greater **relative fitness**: the contribution an individual makes to the gene pool of the next generation *relative to* the contributions of other individuals.

Although we often refer to the relative fitness of a genotype, remember that the entity that is subjected to natural selection is the whole organism, not the underlying genotype. Thus, selection acts more directly on the phenotype than on the genotype; it acts on the genotype indirectly, via how the genotype affects the phenotype.

Directional, Disruptive, and Stabilizing Selection

Natural selection can alter the frequency distribution of heritable traits in three ways, depending on which phenotypes in a population are favored: through directional selection, disruptive selection, and stabilizing selection.

Directional selection occurs when conditions favor individuals exhibiting one extreme of a phenotypic range, thereby shifting a population's frequency curve for the phenotypic

character in one direction or the other (**Figure 23.13a**).

Directional selection is common when a population's environment changes or when members of a population migrate to a new (and different) habitat. For instance, an increase in the relative abundance of large seeds over small seeds led to an increase in beak depth in a population of Galápagos finches (see Figure 23.2).

Disruptive selection (**Figure 23.13b**) occurs when conditions favor individuals at both extremes of a phenotypic range over individuals with intermediate phenotypes. One example is a population of black-bellied seedcracker finches in Cameroon whose members display two distinctly different beak sizes. Small-billed birds feed mainly on soft seeds, whereas large-billed birds specialize in cracking hard seeds. It appears that birds with intermediate-sized bills are relatively inefficient at cracking both types of seeds and thus have lower relative fitness.

Stabilizing selection (**Figure 23.13c**) acts against both extreme phenotypes and favors intermediate variants. This mode of selection reduces variation and tends to maintain the status quo for a particular phenotypic character. For

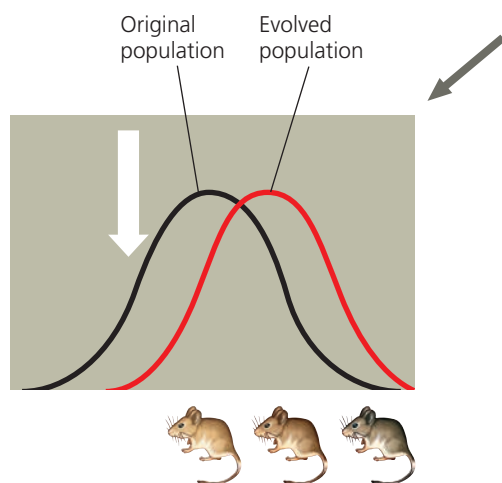
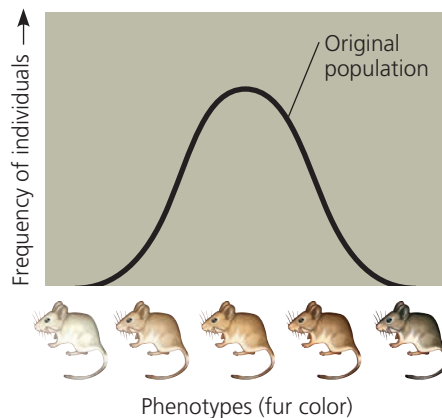
example, the birth weights of most human babies lie in the range of 3–4 kg (6.6–8.8 pounds); babies who are either much smaller or much larger suffer higher rates of mortality.

Regardless of the mode of selection, however, the basic mechanism remains the same. Selection favors individuals whose heritable phenotypic traits provide higher reproductive success than do the traits of other individuals.

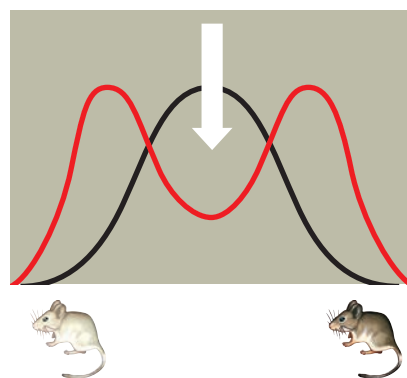
▼ Figure 23.13 Modes of selection.

These cases describe three ways in which a hypothetical deer mouse population with heritable variation in fur coloration might evolve. The graphs show how the frequencies of individuals with different fur colors change over time. The large white arrows symbolize selective pressures against certain phenotypes.

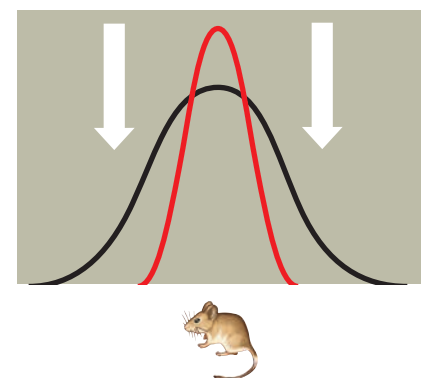
MAKE CONNECTIONS ▶ Review Figure 21.13. Which mode of selection has occurred in soapberry bug populations that feed on the introduced goldenrain tree? Explain.



(a) Directional selection shifts the overall makeup of the population by favoring variants that are at one extreme of the distribution. In this case, lighter mice are selected against because they live among dark rocks, making it harder for them to hide from predators.



(b) Disruptive selection favors variants at both ends of the distribution. These mice have colonized a patchy habitat made up of light and dark rocks, with the result that mice of an intermediate color are selected against.



(c) Stabilizing selection removes extreme variants from the population and preserves intermediate types. If the environment consists of rocks of an intermediate color, both light and dark mice will be selected against.

The Key Role of Natural Selection in Adaptive Evolution

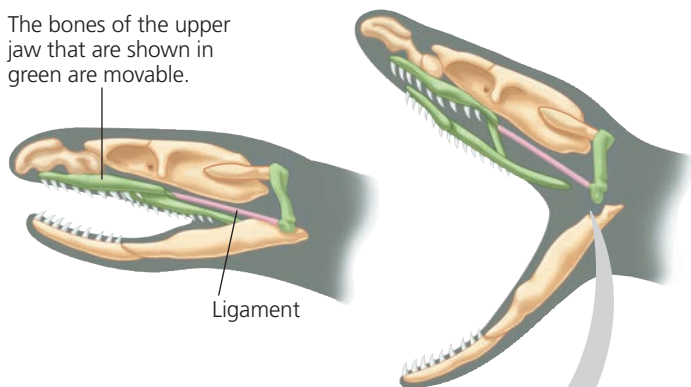
The adaptations of organisms include many striking examples. Certain octopuses, for example, have the ability to change color rapidly, enabling them to blend into different backgrounds. Another example is the remarkable jaws of snakes (**Figure 23.14**), which allow them to swallow prey much larger than their own head (a feat analogous to a person swallowing a whole watermelon). Other adaptations, such as a version of an enzyme that shows improved function in cold environments, may be less visually dramatic but just as important for survival and reproduction.

Such adaptations can arise gradually over time as natural selection increases the frequencies of alleles that enhance survival or reproduction. As the proportion of individuals that have favorable traits increases, the degree to which a species is well suited for life in its environment improves; that is, adaptive evolution occurs. However, the physical and biological components of an organism's environment may change over time. As a result, what constitutes a "good match" between an organism and its environment can be a moving target, making adaptive evolution a continuous, dynamic process. Environmental conditions can also differ from place to place, causing different alleles to be favored in different locations. When this occurs, natural selection can cause the populations of a species to differ genetically from one another.

And what about genetic drift and gene flow? Both can, in fact, increase the frequencies of alleles that enhance survival or reproduction, but neither does so consistently. Genetic drift can cause the frequency of a slightly beneficial allele to

▼ **Figure 23.14** Movable jaw bones in snakes.

The bones of the upper jaw that are shown in green are movable.



The skull bones of most terrestrial vertebrates are relatively rigidly attached to one another, limiting jaw movement. In contrast, most snakes have movable bones in their upper jaw, allowing them to swallow food much larger than their head.



increase, but it also can cause the frequency of such an allele to decrease. Similarly, gene flow may introduce alleles that are advantageous or ones that are disadvantageous. Natural selection is the only evolutionary mechanism that consistently leads to adaptive evolution.



HHMI Video: Got Lactase? The Co-evolution of Genes and Culture



Sexual Selection

Charles Darwin was the first to explore the implications of **sexual selection**, a process in which individuals with certain inherited characteristics are more likely than other individuals of the same sex to obtain mates. Sexual selection can result in **sexual dimorphism**, a difference in secondary sexual characteristics between males and females of the same species (**Figure 23.15**). These distinctions include differences in size, color, ornamentation, and behavior.

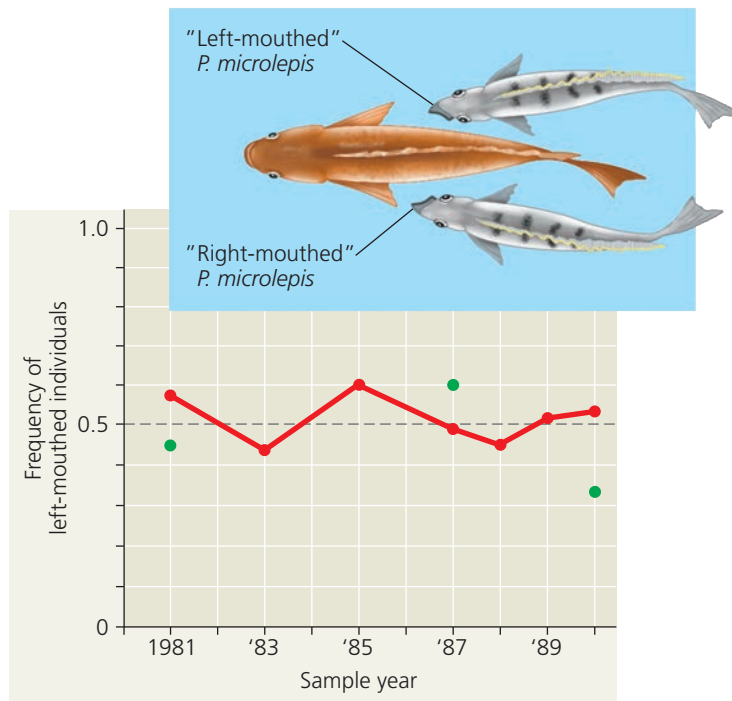
How does sexual selection operate? There are several ways. In **intrasexual selection**, meaning selection within the same sex, individuals of one sex compete directly for mates of the opposite sex. In many species, intrasexual selection occurs among males. For example, a single male may patrol a group of females and prevent other males from mating with them. The patrolling male may defend his status by defeating smaller, weaker, or less fierce males in combat. More often, this male is the psychological victor in ritualized displays that discourage would-be competitors but do not risk injury that would reduce his own fitness (see Figure 52.16). Intrasexual selection also occurs among females in a variety of species, including ring-tailed lemurs and broadnosed pipefish.

In **intersexual selection**, also called *mate choice*, individuals of one sex (usually the females) are choosy in selecting their mates from the other sex. In many cases, the female's choice depends on the showiness of the male's appearance

▼ **Figure 23.15** Sexual dimorphism and sexual selection. A peacock (left) and a peahen (right) show extreme sexual dimorphism. There is intrasexual selection between competing males, followed by intersexual selection when the females choose among the showiest males.



▼ **Figure 23.17 Frequency-dependent selection.** In a population of the scale-eating fish *Perissodus microlepis*, the frequency of left-mouthed individuals (red data points) rises and falls in a regular manner. The frequency of left-mouthed individuals among adults that reproduced was also recorded in three sample years (green data points).



INTERPRET THE DATA ► For 1981, 1987, and 1990, compare the frequency of left-mouthed individuals among breeding adults to the frequency of left-mouthed individuals in the entire population. What do the data indicate about when natural selection favors left-mouthed individuals over right-mouthed individuals (or vice versa)? Explain.

Thus, from year to year, selection favors whichever mouth phenotype is least common. As a result, the frequency of left- and right-mouthed fish oscillates over time, and balancing selection (due to frequency dependence) keeps the frequency of each phenotype close to 50%.

Heterozygote Advantage

If individuals who are heterozygous at a particular locus have greater fitness than do both kinds of homozygotes, they exhibit **heterozygote advantage**. In such a case, natural selection tends to maintain two or more alleles at that locus. Note that heterozygote advantage is defined in terms of *genotype*, not *phenotype*. Thus, whether heterozygote advantage represents stabilizing or directional selection depends on the relationship between the genotype and the phenotype. For example, if the phenotype of a heterozygote is intermediate to the phenotypes of both homozygotes, heterozygote advantage is a form of stabilizing selection.

An example of heterozygote advantage occurs at the locus in humans that codes for the β polypeptide subunit of hemoglobin, the oxygen-carrying protein of red blood cells. In homozygous individuals, a recessive allele at that locus causes

sickle-cell disease. The red blood cells of people with sickle-cell disease become distorted in shape, or *sickled*, under low-oxygen conditions (see Figure 5.19), as occurs in the capillaries. These sickled cells can clump together and block the flow of blood in the capillaries, damaging organs such as the kidney, heart, and brain. Although some red blood cells become sickled in heterozygotes, not enough become sickled to cause sickle-cell disease.

Heterozygotes for the sickle-cell allele are protected against the most severe effects of malaria, a disease caused by a parasite that infects red blood cells (see Figure 28.16). One reason for this partial protection is that the body destroys sickled red blood cells rapidly, killing the parasites they harbor. Malaria is a major killer in some tropical regions. In such regions, selection favors heterozygotes over homozygous dominant individuals, who are more vulnerable to the effects of malaria, and also over homozygous recessive individuals, who develop sickle-cell disease. As described in **Figure 23.18**, these selective pressures have caused the frequency of the sickle-cell allele to reach relatively high levels in areas where the malaria parasite is common.

Why Natural Selection Cannot Fashion Perfect Organisms

Though natural selection leads to adaptation, nature abounds with examples of organisms that are less than ideally suited for their lifestyles. There are several reasons why.

1. Selection can act only on existing variations.

Natural selection favors only the fittest phenotypes among those currently in the population, which may not be the ideal traits. New advantageous alleles do not arise on demand.

2. Evolution is limited by historical constraints.

Each species has a legacy of descent with modification from ancestral forms. Evolution does not scrap the ancestral anatomy and build each new complex structure from scratch; rather, evolution co-opts existing structures and adapts them to new situations. We could imagine that if a terrestrial animal were to adapt to an environment in which flight would be advantageous, it might be best just to grow an extra pair of limbs that would serve as wings. However, evolution does not work this way; instead, it operates on the traits an organism already has. Thus, in birds and bats, an existing pair of limbs took on new functions for flight as these organisms evolved from nonflying ancestors.

3. Adaptations are often compromises.

Each organism must do many different things. A seal spends part of its time on rocks; it could probably walk better if it had legs instead of flippers, but then it would not swim nearly as well. We humans owe much of our versatility and athleticism to our prehensile hands and flexible limbs, but these also make us prone to sprains, torn ligaments, and dislocations: Structural reinforcement has been compromised for agility.

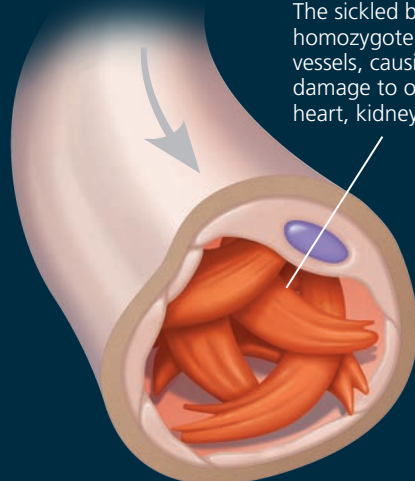


Infected mosquitoes spread malaria when they bite people. (See Figure 28.16.)

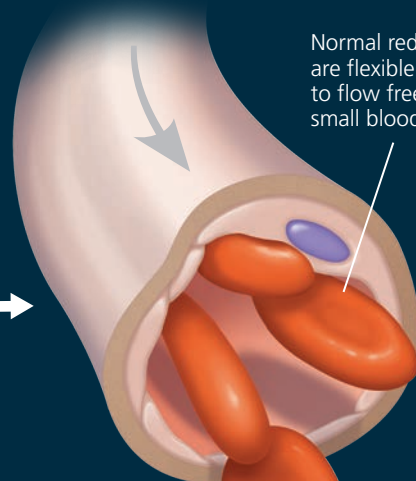
Effects on Individual Organisms

- The formation of sickled red blood cells causes homozygotes with two copies of the sickle-cell allele to have sickle-cell disease.
- Some sickling also occurs in heterozygotes, but not enough to cause the disease; they have sickle-cell trait. (See Figure 14.17.)

The sickled blood cells of a homozygote block small blood vessels, causing great pain and damage to organs such as the heart, kidney, and brain.

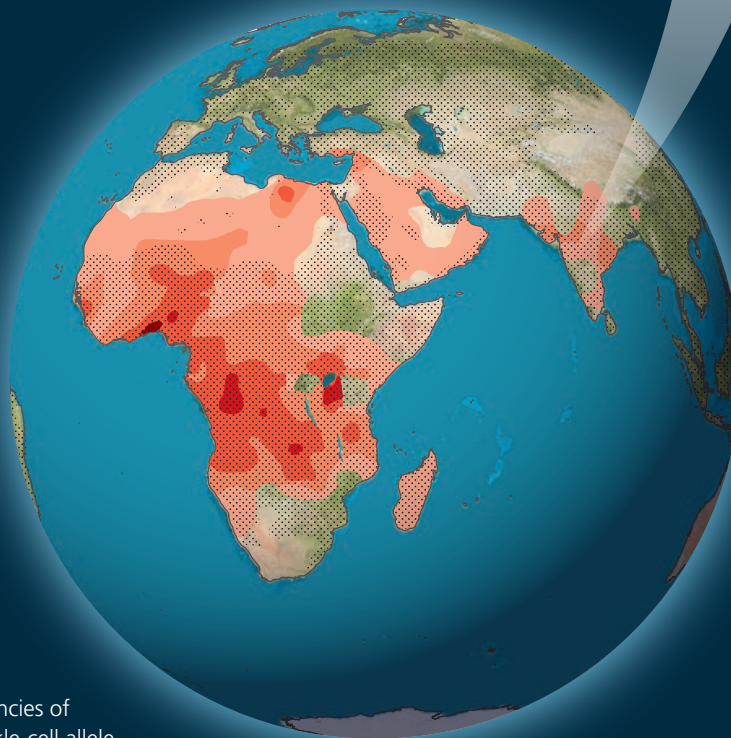


Normal red blood cells are flexible and are able to flow freely through small blood vessels.



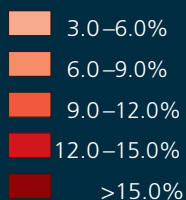
Evolution in Populations

- Homozygotes with two sickle-cell alleles are strongly selected against because of mortality caused by sickle-cell disease. In contrast, heterozygotes experience few harmful effects from sickling yet are more likely to survive malaria than are homozygotes.
- In regions where malaria is common, the net effect of these opposing selective forces is heterozygote advantage. This has caused evolutionary change in populations—the products of which are the areas of relatively high frequencies of the sickle-cell allele shown in the map below.



Key

Frequencies of the sickle-cell allele



Distribution of malaria caused by *Plasmodium falciparum* (a parasitic unicellular eukaryote)

MAKE CONNECTIONS > In a region free of malaria, would individuals who are heterozygous for the sickle-cell allele be selected for or selected against? Explain.

4. Chance, natural selection, and the environment interact. Chance events can affect the subsequent evolutionary history of populations. For instance, when a storm blows insects or birds hundreds of kilometers over an ocean to an island, the wind does not necessarily transport those individuals that are best suited to the new environment. Thus, not all alleles present in the founding population's gene pool are better suited to the new environment than the alleles that are "left behind." In addition, the environment at a particular location may change unpredictably from year to year, again limiting the extent to which adaptive evolution results in organisms being well suited for current environmental conditions.

With these four constraints, evolution does not tend to craft perfect organisms. Natural selection operates on a "better

than" basis. We can, in fact, see evidence for evolution in the many imperfections of the organisms it produces.

CONCEPT CHECK 23.4

1. What is the relative fitness of a sterile mule? Explain.
2. Explain why natural selection is the only evolutionary mechanism that consistently leads to adaptive evolution in a population.
3. **VISUAL SKILLS** > Consider a population in which heterozygotes at a certain locus have an extreme phenotype (such as being larger than homozygotes) that confers a selective advantage. Compare this description to the models of selection modes shown in Figure 23.13. Does this situation represent directional, disruptive, or stabilizing selection? Explain your answer.

For suggested answers, see Appendix A.

23 Chapter Review



Go to **MasteringBiology™** for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

SUMMARY OF KEY CONCEPTS

CONCEPT 23.1

Genetic variation makes evolution possible (pp. 541–543)

- **Genetic variation** refers to genetic differences among individuals within a population.
- The nucleotide differences that provide the basis of genetic variation originate when mutation and gene duplication produce new alleles and new genes. New genetic variants are produced rapidly in organisms with short generation times. In sexually reproducing organisms, most of the genetic differences among individuals result from crossing over, the independent assortment of chromosomes, and fertilization.

? Typically, most of the nucleotide variability that occurs within a genetic locus does not affect the phenotype. Explain why.



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax

CONCEPT 23.2

The Hardy-Weinberg equation can be used to test whether a population is evolving (pp. 543–547)

- A **population**, a localized group of organisms belonging to one species, is united by its **gene pool**, the aggregate of all the alleles in the population.
- For a population in **Hardy-Weinberg equilibrium**, the allele and genotype frequencies will remain constant if the population is large, mating is random, mutation is negligible, there is no gene flow, and there is no natural selection. For such a population, if p and q represent the frequencies of the only two possible alleles at a particular locus, then p^2 is the frequency of one kind of homozygote, q^2 is the frequency of the other kind of homozygote, and $2pq$ is the frequency of the heterozygous genotype.

? Is it circular reasoning to calculate p and q from observed genotype frequencies and then use those values of p and q to test if the population is in Hardy-Weinberg equilibrium? Explain your answer.

CONCEPT 23.3

Natural selection, genetic drift, and gene flow can alter allele frequencies in a population (pp. 547–551)

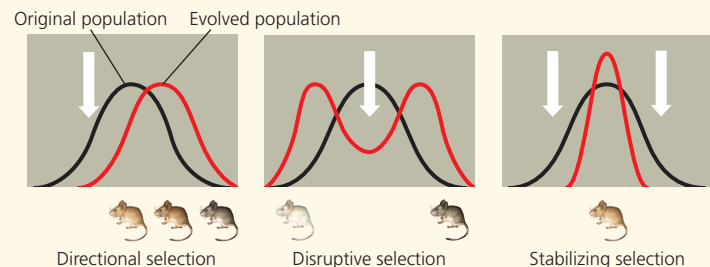
- In natural selection, individuals that have certain inherited traits tend to survive and reproduce at higher rates than other individuals *because of* those traits.
- In **genetic drift**, chance fluctuations in allele frequencies over generations tend to reduce genetic variation.
- **Gene flow**, the transfer of alleles between populations, tends to reduce genetic differences between populations over time.

? Would two small, geographically isolated populations in very different environments be likely to evolve in similar ways? Explain.

CONCEPT 23.4

Natural selection is the only mechanism that consistently causes adaptive evolution (pp. 551–558)

- One organism has greater **relative fitness** than another organism if it leaves more fertile descendants. The modes of natural selection differ in their effect on phenotype:



- Unlike genetic drift and gene flow, natural selection consistently increases the frequencies of alleles that enhance survival and reproduction, thus improving the degree to which organisms are well-suited for life in their environment.
- **Sexual selection** can result in secondary sex characteristics that can give individuals advantages in mating.

- **Balancing selection** occurs when natural selection maintains two or more forms in a population.
- There are constraints to evolution: Natural selection can act only on available variation; structures result from modified ancestral anatomy; adaptations are often compromises; and chance, natural selection, and the environment interact.

? How might secondary sex characteristics in males differ from those in females in a species in which females compete for mates?

TEST YOUR UNDERSTANDING



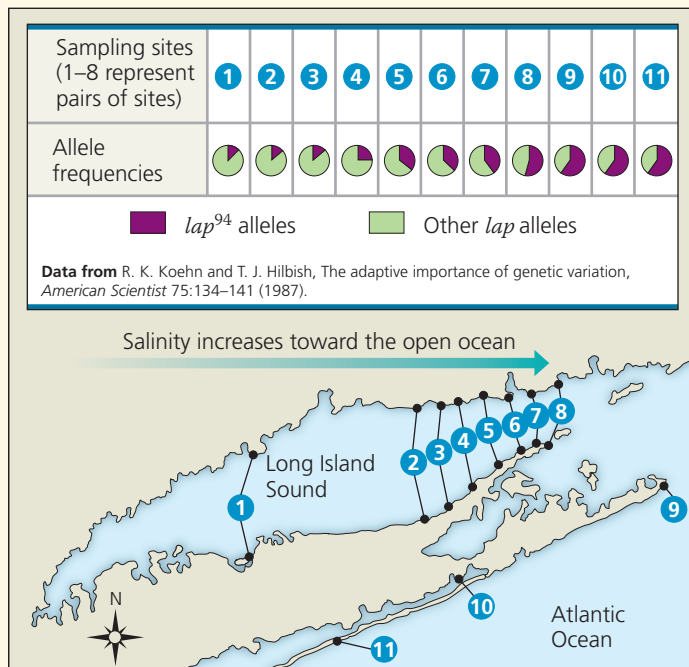
Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

6. **EVOLUTION CONNECTION** Using at least two examples, explain how the process of evolution is revealed by the imperfections of living organisms.



PRACTICE TEST
goo.gl/iAsVgI

7. **SCIENTIFIC INQUIRY • INTERPRET THE DATA** Researchers studied genetic variation in the marine mussel *Mytilus edulis* around Long Island, New York. They measured the frequency of a particular allele (*lap*⁹⁴) for an enzyme involved in regulating the mussel's internal saltwater balance. The researchers presented their data as a series of pie charts linked to sampling sites within Long Island Sound, where the salinity is highly variable, and along the coast of the open ocean, where salinity is constant. (a) Create a data table for the 11 sampling sites by estimating the frequency of *lap*⁹⁴ from the pie charts. (Hint: Think of each



pie chart as a clock face to help you estimate the proportion of the shaded area.) (b) Graph the frequencies for sites 1–8 to show how the frequency of this allele changes with increasing salinity in Long Island Sound (from southwest to northeast). Evaluate how the data from sites 9–11 compare with the data from the sites within the Sound. (c) Considering the various mechanisms that can alter allele frequency, construct a hypothesis that explains the patterns you observe in the data and that accounts for the following observations: (1) The *lap*⁹⁴ allele helps mussels maintain osmotic balance in water with a high salt concentration but is costly to use in less salty water; and (2) mussels produce larvae that can disperse long distances before they settle on rocks and grow into adults.

8. **WRITE ABOUT A THEME: ORGANIZATION** Heterozygotes at the sickle-cell locus produce both normal and abnormal (sickle-cell) hemoglobin (see Concept 14.4). When hemoglobin molecules are packed into a heterozygote's red blood cells, some cells receive relatively large quantities of abnormal hemoglobin, making these cells prone to sickling. In a short essay (approximately 100–150 words), explain how these molecular and cellular events lead to emergent properties at the individual and population levels of biological organization.

9. SYNTHESIZE YOUR KNOWLEDGE



This kettle lake formed 14,000 years ago when a glacier that covered the surrounding area melted. Initially devoid of animal life, over time the lake was colonized by invertebrates and other animals. Hypothesize how mutation, natural selection, genetic drift, and gene flow may have affected populations that colonized the lake.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Species and Speciation

24



▲ **Figure 24.1** How did this flightless bird come to live on the isolated Galápagos Islands?

KEY CONCEPTS

- 24.1** The biological species concept emphasizes reproductive isolation
- 24.2** Speciation can take place with or without geographic separation
- 24.3** Hybrid zones reveal factors that cause reproductive isolation
- 24.4** Speciation can occur rapidly or slowly and can result from changes in few or many genes

▼ **Galápagos giant tortoise, another species unique to the islands**



That “Mystery of Mysteries”

When Darwin came to the Galápagos Islands, he noted that these volcanic islands were teeming with plants and animals found nowhere else in the world (**Figure 24.1**). Later he realized that these species had formed relatively recently. He wrote in his diary, “Both in space and time, we seem to be brought somewhat near to that great fact—that mystery of mysteries—the first appearance of new beings on this Earth.”

The “mystery of mysteries” that captivated Darwin is **speciation**, the process by which one species splits into two or more species. Speciation fascinated Darwin (and many biologists since) because it has produced the tremendous diversity of life, repeatedly yielding new species that differ from existing ones. Later, Darwin realized that speciation also helps to explain the many features that organisms share (the unity of life): When one species splits into two, the species that result share many characteristics because they are descended from this common ancestor. At the DNA sequence level, for example, such similarities indicate that the flightless cormorant (*Phalacrocorax harrisi*) in **Figure 24.1** is closely related to flying cormorants found in the Americas. This suggests that the flightless cormorant originated from an ancestral cormorant species that flew from the mainland to the Galápagos.

Speciation also forms a conceptual bridge between **microevolution**, changes over time in allele frequencies in a population, and **macroevolution**, the broad pattern of evolution above the species level. An example of

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Get Ready for This Chapter

macroevolutionary change is the origin of new groups of organisms, such as mammals or flowering plants, through a series of speciation events. We examined microevolutionary mechanisms in Chapter 23, and we'll turn to macroevolution in Chapter 25. In this chapter, we'll explore the "bridge" between microevolution and macroevolution—the mechanisms by which new species originate from existing ones. First, let's establish what we actually mean by a "species."



HHMI Video: The Origin of Species:
The Beak of the Finch



CONCEPT 24.1

The biological species concept emphasizes reproductive isolation

The word *species* is Latin for "kind" or "appearance." In daily life, we commonly distinguish between various "kinds" of organisms—dogs and cats, for instance—based on differences in their appearance. But are organisms truly divided into the discrete units we call species? To answer this question, biologists compare not only the morphology (body form) of different groups of organisms but also less obvious differences in physiology, biochemistry, and DNA sequences. The results generally confirm that morphologically distinct species are indeed discrete groups, differing in many ways besides their body forms.

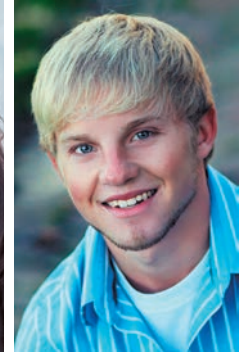
The Biological Species Concept

The primary definition of species used in this textbook is the **biological species concept**. According to this concept, a **species** is a group of populations whose members have the potential to interbreed in nature and produce viable, fertile offspring—but do not produce viable, fertile offspring with members of other such groups (Figure 24.2). Thus, the members of a biological species are united by being reproductively compatible, at least potentially. All human beings, for example, belong to the same species. A businesswoman in Manhattan may be unlikely to meet a dairy farmer in Mongolia, but if the two should happen to meet and mate, they could have viable babies who develop into fertile adults. In contrast, humans and chimpanzees remain distinct biological species, even where they live in the same region, because many factors keep them from interbreeding and producing fertile offspring.

What holds the gene pool of a species together, causing its members to resemble each other more than they resemble members of other species? Recall the evolutionary mechanism of *gene flow*, the transfer of alleles between populations (see Concept 23.3). Typically, gene flow occurs between the different populations of a species. This ongoing exchange of alleles tends to hold the populations together genetically. But as we'll explore in this chapter, a reduction or lack of gene flow can play a key role in the formation of new species.



(a) **Similarity between different species.** The eastern meadowlark (*Sturnella magna*, left) and the western meadowlark (*Sturnella neglecta*, right) have similar body shapes and colorations. Nevertheless, they are distinct biological species because their songs and other behaviors are different enough to prevent interbreeding should they meet in the wild.



(b) **Diversity within a species.** As diverse as we may be in appearance, all humans belong to a single biological species (*Homo sapiens*), defined by our capacity to interbreed successfully.

▲ Figure 24.2 The biological species concept is based on the potential to interbreed, not on physical similarity.

Reproductive Isolation

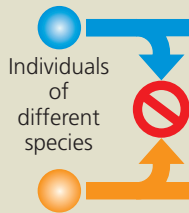
Because biological species are defined in terms of reproductive compatibility, the formation of a new species hinges on **reproductive isolation**—the existence of biological factors (barriers) that impede members of two species from interbreeding and producing viable, fertile offspring. Such barriers block gene flow between the species and limit the formation of **hybrids**, offspring that result from an interspecific mating. Although a single barrier may not prevent all gene flow, a combination of several barriers can effectively isolate a species' gene pool.

Clearly, a fly cannot mate with a frog or a fern, but the reproductive barriers between more closely related species are not so obvious. As described in Figure 24.3,

▼ Figure 24.3 Exploring Reproductive Barriers

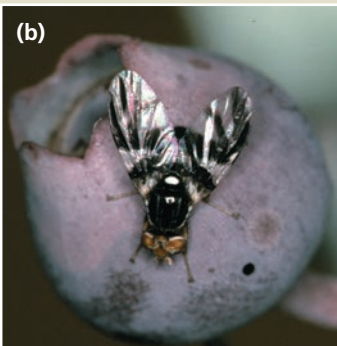
Prezygotic barriers impede mating or hinder fertilization if mating does occur

Habitat Isolation



Two species that occupy different habitats within the same area may encounter each other rarely, if at all, even though they are not isolated by obvious physical barriers, such as mountain ranges.

Example: These two fly species in the genus *Rhagoletis* occur in the same geographic areas, but the apple maggot fly (*Rhagoletis pomonella*) feeds and mates on hawthorns and apples (a) while its close relative, the blueberry maggot fly (*R. mendax*), mates and lays its eggs only on blueberries (b).



Temporal Isolation



Species that breed during different times of the day, different seasons, or different years cannot mix their gametes.

Example: In North America, the geographic ranges of the western spotted skunk (*Spilogale gracilis*) (c) and the eastern spotted skunk (*Spilogale putorius*) (d) overlap, but *S. gracilis* mates in late summer and *S. putorius* mates in late winter.



Behavioral Isolation



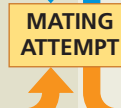
Courtship rituals that attract mates and other behaviors unique to a species are effective reproductive barriers, even between closely related species. Such behavioral rituals enable *mate recognition*—a way to identify potential mates of the same species.

Example: Blue-footed boobies, inhabitants of the Galápagos, mate only after a courtship display unique to their species. Part of the “script” calls for the male to high-step (e), a behavior that calls the female’s attention to his bright blue feet.



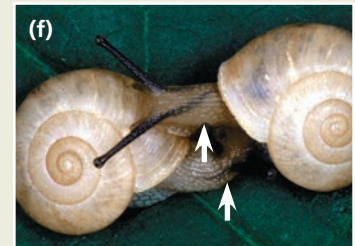
 **Video: Blue-Footed Boobies Courtship Ritual**

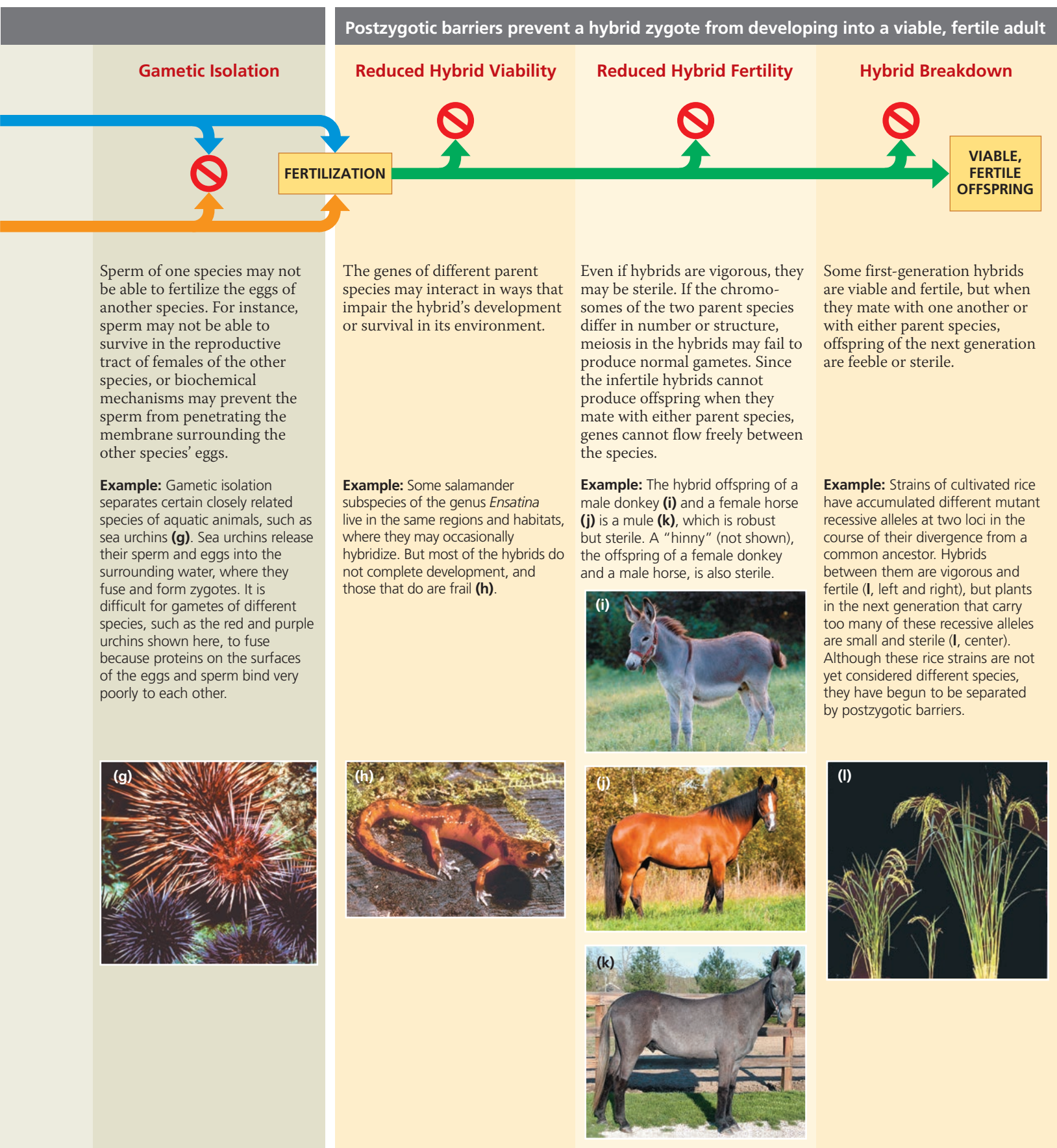
Mechanical Isolation



Mating is attempted, but morphological differences prevent its successful completion.

Example: The shells of two species of snails in the genus *Bradybaena* spiral in different directions: Moving inward to the center, one spirals in a counterclockwise direction (f, left), the other in a clockwise direction (f, right). As a result, the snails’ genital openings (indicated by arrows) are not aligned, and mating cannot be completed.





these barriers can be classified according to whether they contribute to reproductive isolation before or after fertilization. **Prezygotic barriers** (“before the zygote”) block fertilization from occurring. Such barriers typically act in one of three ways: by impeding members of different species from attempting to mate, by preventing an attempted mating from being completed successfully, or by hindering fertilization if mating is completed successfully. If a sperm cell from one species overcomes prezygotic barriers and fertilizes an ovum from another species, a variety of **postzygotic barriers** (“after the zygote”) may contribute to reproductive isolation after the hybrid zygote is formed. Developmental errors may reduce survival among hybrid embryos. Or problems after birth may cause hybrids to be infertile or decrease their chance of surviving long enough to reproduce.

Limitations of the Biological Species Concept

One strength of the biological species concept is that it directs our attention to a way by which speciation can occur: by the evolution of reproductive isolation. However, the number of species to which this concept can be usefully applied is limited. There is, for example, no way to evaluate the reproductive isolation of fossils. The biological species concept also does not apply to organisms that reproduce asexually all or most of the time, such as prokaryotes. (Many prokaryotes do transfer genes among themselves, as we will discuss in Concept 27.2, but this is not part of their reproductive process.) Furthermore, in the biological species concept, species are designated by the *absence* of gene flow. But there are many pairs of species that are morphologically and ecologically distinct, and yet gene flow occurs between them. An example is the grizzly bear (*Ursus arctos*) and polar bear (*Ursus maritimus*), whose hybrid offspring have been dubbed “grolar bears” (Figure 24.4). As we’ll discuss, natural selection can cause such species to remain distinct even though some gene flow occurs between them. Because of the limitations to the biological species concept, alternative species concepts are useful in certain situations.

Other Definitions of Species

While the biological species concept emphasizes the *separateness* of different species due to reproductive barriers, several other definitions emphasize the *unity within* a species. For example, the **morphological species concept** distinguishes a species by body shape and other structural features. The morphological species concept can be applied to asexual and sexual organisms, and it can be useful even without information on the extent of gene flow. In practice, scientists often distinguish species using morphological criteria. A disadvantage of this approach, however, is that it relies on subjective criteria; researchers may disagree on which structural features distinguish a species.



◀ Grizzly bear (*U. arctos*)

▼ Polar bear (*U. maritimus*)



◀ Hybrid “grolar bear”

▲ **Figure 24.4** Hybridization between two species of bears in the genus *Ursus*.

The **ecological species concept** defines a species in terms of its ecological niche, the sum of how members of the species interact with the nonliving and living parts of their environment (see Concept 54.1). For example, two species of oak trees might differ in their size or in their ability to tolerate dry conditions, yet still occasionally interbreed. Because they occupy different ecological niches, these oaks would be considered separate species even though they are connected by some gene flow. Unlike the biological species concept, the ecological species concept can accommodate asexual as well as sexual species. It also emphasizes the role of disruptive natural selection as organisms adapt to different environments.

In addition to those discussed here, more than 20 other species definitions have been proposed. The usefulness of each definition depends on the situation and the research questions being asked. For our purposes of studying how species originate, the biological species concept, with its focus on reproductive barriers, is particularly helpful.

CONCEPT CHECK 24.1

1. (a) Which species concept(s) could you apply to both asexual and sexual species? (b) Which would be most useful for identifying species in the field? Explain.
2. **WHAT IF? >** Suppose two bird species live in a forest and are not known to interbreed. One species feeds and mates in the treetops and the other on the ground. But in captivity, the birds can interbreed and produce viable, fertile offspring. What type of reproductive barrier most likely keeps these species separate in nature? Explain.

For suggested answers, see Appendix A.

CONCEPT 24.2

Speciation can take place with or without geographic separation

Having discussed what constitutes a unique species, let's return to the process by which such species arise from existing species. We'll describe this process by focusing on the geographic setting in which gene flow is interrupted between populations of the existing species—in allopatric speciation the populations are geographically isolated, while in sympatric speciation they are not (Figure 24.5).

Allopatric ("Other Country") Speciation

In **allopatric speciation** (from the Greek *allos*, other, and *patra*, homeland), gene flow is interrupted when a population is divided into geographically isolated subpopulations. For example, the water level in a lake may subside, resulting in two or more smaller lakes that are now home to separated populations (see Figure 24.5a). Or a river may change course and divide a population of animals that cannot cross it. Allopatric speciation can also occur without geologic remodeling, such as when individuals colonize a remote area and their descendants become geographically isolated from the parent population. The flightless cormorant shown in Figure 24.1 probably originated in this way from an ancestral flying species that reached the Galápagos Islands.

The Process of Allopatric Speciation

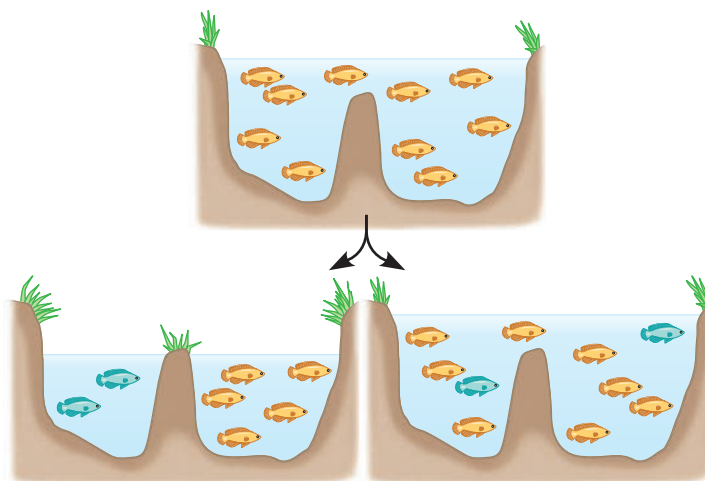
How formidable must a geographic barrier be to promote allopatric speciation? The answer depends on the ability of the organisms to move about. Birds, mountain lions, and coyotes can cross rivers and canyons—as can the windblown pollen of pine trees and the seeds of some flowering plants. In contrast,

small rodents may find a wide river or deep canyon a formidable barrier.

Once geographic isolation has occurred, the separated gene pools may diverge. Different mutations arise, and natural selection and genetic drift may alter allele frequencies in different ways in the separated populations. Reproductive isolation may then evolve as a by-product of the genetic divergence that results from selection or drift.

Figure 24.6 describes an example. On Andros Island, in the Bahamas, populations of the mosquitofish *Gambusia hubbsi* colonized a series of ponds that later became isolated from one another. Genetic analyses indicate that little or no gene flow currently occurs between the ponds. The environments of these ponds are very similar except that some contain predatory fishes, while others do not. In ponds with predatory fishes, selection has favored the evolution of a mosquitofish body shape that enables rapid bursts of speed (Figure 24.6). In ponds without predatory fishes, selection has favored a different body shape, one that improves the ability to swim for long periods of time. How have these different selective pressures affected the evolution of reproductive barriers? Researchers studied this question by bringing together mosquitofish from the two types of ponds. They found that female mosquitofish prefer to mate with males whose body shape is similar to their own. This preference establishes a behavioral barrier to reproduction between mosquitofish from ponds with predators and those

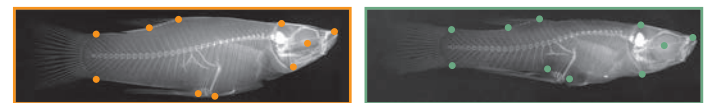
▼ **Figure 24.5** The geography of speciation.



- (a) **Allopatric speciation.** A population forms a new species while geographically isolated from its parent population.
- (b) **Sympatric speciation.** A subset of a population forms a new species without geographic separation.

▼ **Figure 24.6** Evolution in mosquitofish populations.

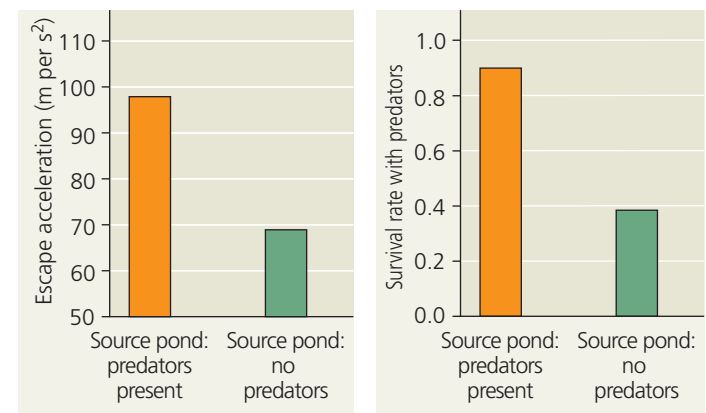
Different body shapes have evolved in mosquitofish populations from ponds with and without predators. These differences affect how quickly the fish can accelerate to escape and their survival rate when exposed to predators.



In ponds with predatory fishes, the mosquitofish's head is streamlined and the tail is powerful, enabling rapid bursts of speed.

In ponds without predatory fishes, mosquitofish have a different body shape that favors long, steady swimming.

(a) Differences in body shape



(b) Differences in escape acceleration and survival

from ponds without predators. Thus, as a by-product of selection for avoiding predators, reproductive barriers have formed in these allopatric populations.

Evidence of Allopatric Speciation

Many studies provide evidence that speciation can occur in allopatric populations. For example, laboratory studies show that reproductive barriers can develop when populations are isolated experimentally and subjected to different environmental conditions (Figure 24.7).

Field studies indicate that allopatric speciation also can occur in nature. Consider the 30 species of snapping shrimp in the genus *Alpheus* that live off the Isthmus of Panama, the land bridge that connects South and North America (Figure 24.8). Fifteen of these species live on the Atlantic side of the isthmus, while the other 15 live on the Pacific side. Before the isthmus formed, gene flow could occur between the Atlantic and Pacific populations of snapping shrimp. Did the species on different sides of the isthmus originate by allopatric speciation? Morphological and genetic data group these shrimp into 15 pairs of *sister species*, pairs whose member species are each other's closest relative.

Figure 24.8 Allopatric speciation in snapping shrimp (*Alpheus*). The shrimp pictured are just 2 of the 15 pairs of sister species that arose as populations were divided by the formation of the Isthmus of Panama. The color-coded type indicates the sister species.

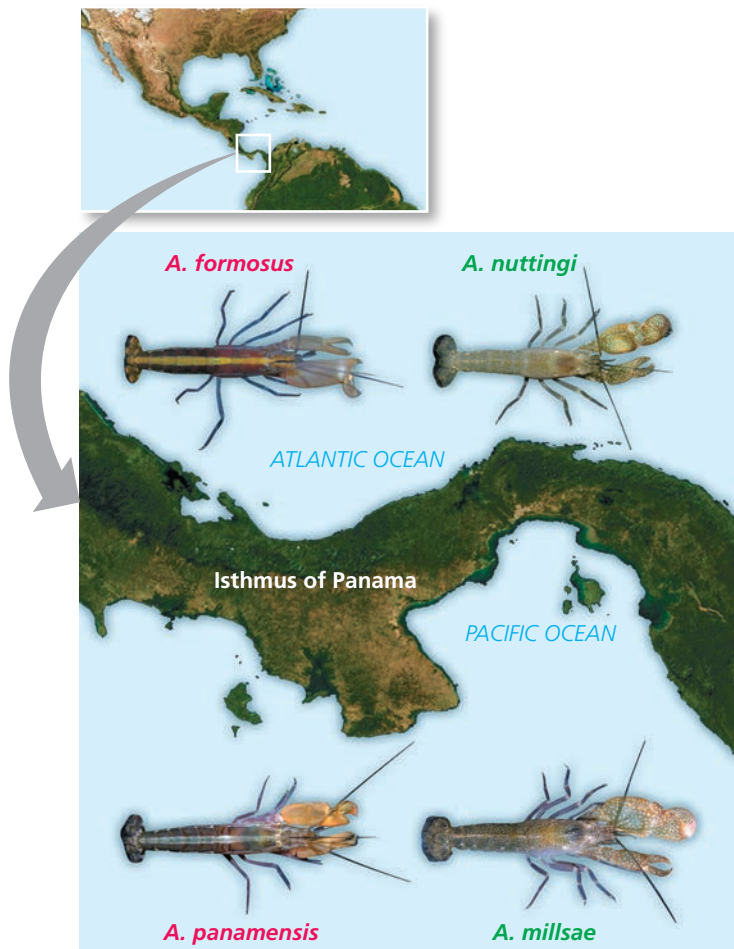
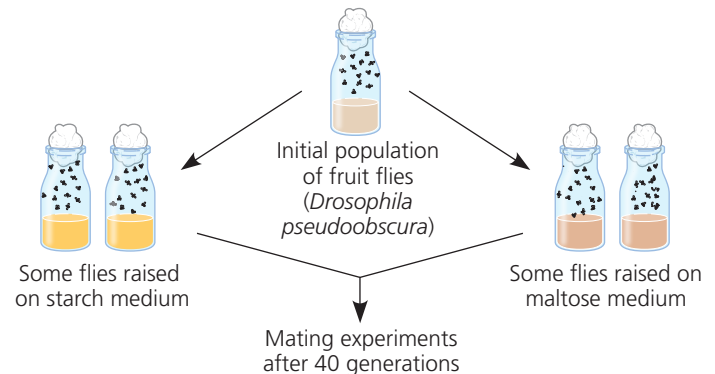


Figure 24.7

Inquiry Can divergence of allopatric populations lead to reproductive isolation?

Experiment A researcher divided a laboratory population of the fruit fly *Drosophila pseudoobscura*, raising some flies on a starch medium and others on a maltose medium. After one year (about 40 generations), natural selection resulted in divergent evolution: Populations raised on starch digested starch more efficiently, while those raised on maltose digested maltose more efficiently. The researcher then put flies from the same or different populations in mating cages and measured mating frequencies. All flies used in the mating preference tests were reared for one generation on a standard cornmeal medium.



Results Mating patterns among populations of flies raised on different media are shown below. When flies from “starch populations” were mixed with flies from “maltose populations,” the flies tended to mate with like partners. But in the control group (shown on the right), flies from different populations adapted to starch were about as likely to mate with each other as with flies from their own population; similar results were obtained for control groups adapted to maltose.

		Female	
		Starch	Maltose
Male	Starch	22	9
	Maltose	8	20

Number of matings in experimental group

		Female	
		Starch population 1	Starch population 2
Male	Starch population 1	18	15
	Starch population 2	12	15

Number of matings in control group

Conclusion In the experimental group, the strong preference of “starch flies” and “maltose flies” to mate with like-adapted flies indicates that a reproductive barrier was forming between these fly populations. Although this barrier was not absolute (some mating between starch flies and maltose flies did occur), after 40 generations reproductive isolation appeared to be increasing. This barrier may have been caused by differences in courtship behavior that arose as an incidental by-product of differing selective pressures as these allopatric populations adapted to different sources of food.

Data from D. M. B. Dodd, Reproductive isolation as a consequence of adaptive divergence in *Drosophila pseudoobscura*, *Evolution* 43:1308–1311 (1989).

WHAT IF? > Why were all flies used in the mating preference tests reared on a standard medium (rather than on starch or maltose)?

In each of these 15 pairs, one of the sister species lives on the Atlantic side of the isthmus, while the other lives on the Pacific side. This fact strongly suggests that the two species arose as a consequence of geographic separation. Furthermore, genetic analyses indicate that the *Alpheus* species originated from 9 to 3 million years ago, with the sister species that live in the deepest water diverging first. These divergence times are consistent with geologic evidence that the isthmus formed gradually, starting 10 million years ago, and closing completely about 3 million years ago.

The importance of allopatric speciation is also suggested by the fact that regions that are isolated or highly subdivided by barriers typically have more species than do otherwise similar regions that lack such features. For example, many unique plants and animals are found on the geographically isolated Hawaiian Islands (we'll return to the origin of Hawaiian species in Concept 25.4). Field studies also show that reproductive isolation between two populations generally increases as the geographic distance between them increases, a finding consistent with allopatric speciation. In the **Scientific Skills Exercise**, you will analyze data from one such study that

examined reproductive isolation in geographically separated salamander populations.

Note that while geographic isolation prevents interbreeding between members of allopatric populations, physical separation is not a biological barrier to reproduction. Biological reproductive barriers such as those described in Figure 24.3 are intrinsic to the organisms themselves. Hence, it is biological barriers that can prevent interbreeding when members of different populations come into contact with one another.

Sympatric ("Same Country") Speciation

In **sympatric speciation** (from the Greek *syn*, together), speciation occurs in populations that live in the same geographic area (see Figure 24.5b). How can reproductive barriers form between sympatric populations while their members remain in contact with each other? Although such contact (and the ongoing gene flow that results) makes sympatric speciation less common than allopatric speciation, sympatric speciation can occur if gene flow is reduced by such factors as polyploidy, sexual selection, and habitat differentiation. (Note that these factors can also promote allopatric speciation.)

SCIENTIFIC SKILLS EXERCISE

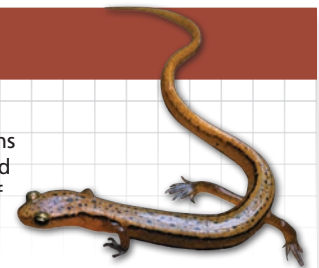
Identifying Independent and Dependent Variables, Making a Scatter Plot, and Interpreting Data

Does Distance Between Salamander Populations Increase Their Reproductive Isolation? Allopatric speciation begins when populations become geographically isolated, preventing mating between individuals in different populations and thus stopping gene flow. It is logical that as distance between populations increases, so will their degree of reproductive isolation. To test this hypothesis, researchers studied populations of the dusky salamander (*Desmognathus ochrophaeus*) living on different mountain ranges in the southern Appalachians.

How the Experiment Was Done The researchers tested the reproductive isolation of pairs of salamander populations by leaving one male and one female together and later checking the females for the presence of sperm. Four mating combinations were tested for each pair of populations (A and B)—two *within* the same population (female A with male A and female B with male B) and two *between* populations (female A with male B and female B with male A).

Data from the Experiment The researchers used an index of reproductive isolation that ranged from a value of 0 (no isolation) to a value of 2 (full isolation). The proportion of successful matings for each mating combination was measured, with 100% success = 1 and no success = 0. The reproductive isolation value for two populations is the sum of the proportion of successful matings of each type within populations (AA + BB) minus the sum of the proportion of successful

matings of each type between populations (AB + BA). The table provides distance and reproductive isolation data for 27 pairs of dusky salamander populations.



INTERPRET THE DATA

1. State the researchers' hypothesis, and identify the independent and dependent variables in this study. Explain why the researchers used four mating combinations for each pair of populations.
2. Calculate the value of the reproductive isolation index if (a) *all* of the matings within a population were successful, but *none* of the matings between populations were successful; (b) salamanders are equally successful in mating with members of their own population and members of another population.
3. Make a scatter plot to help you visualize any patterns that might indicate a relationship between the variables. Plot the independent variable on the x-axis and the dependent variable on the y-axis. (For additional information about graphs, see the Scientific Skills Review in Appendix F and the Study Area of MasteringBiology.)
4. Interpret your graph by (a) explaining in words any pattern indicating a possible relationship between the variables and (b) hypothesizing the possible cause of such a relationship.

Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Data from S. G. Tilley, A. Verrell, and S. J. Arnold, Correspondence between sexual isolation and allozyme differentiation: a test in the salamander *Desmognathus ochrophaeus*, *Proceedings of the National Academy of Sciences USA* 87:2715–2719 (1990).

Geographic Distance (km)	15	32	40	47	42	62	63	81	86	107	107	115	137	147
Reproductive Isolation Value	0.32	0.54	0.50	0.50	0.82	0.37	0.67	0.53	1.15	0.73	0.82	0.81	0.87	0.87
Distance (continued)	137	150	165	189	219	239	247	53	55	62	105	179	169	
Isolation (continued)	0.50	0.57	0.91	0.93	1.5	1.22	0.82	0.99	0.21	0.56	0.41	0.72	1.15	

Polyploidy

A species may originate from an accident during cell division that results in extra sets of chromosomes, a condition called **polyploidy**. Polyploid speciation occasionally occurs in animals; for example, the gray tree frog *Hyla versicolor* (see Figure 23.16) is thought to have originated in this way. However, polyploidy is far more common in plants. In fact, botanists estimate that more than 80% of the plant species alive today are descended from ancestors that formed by polyploid speciation.

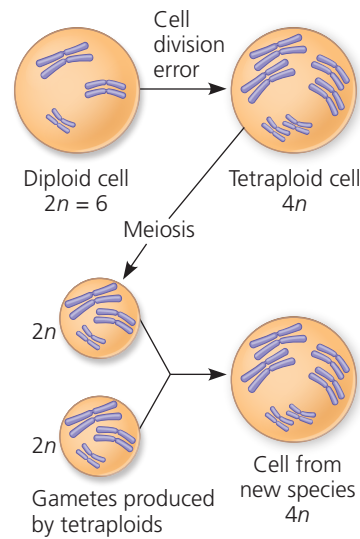
Two distinct forms of polyploidy have been observed in plant (and a few animal) populations. An **autopolyploid** (from the Greek *autos*, self) is an individual that has more than two chromosome sets that are all derived from a single species. In plants, for example, a failure of cell division could double a cell's chromosome number from the original number ($2n$) to a tetraploid number ($4n$) (Figure 24.9).

A tetraploid can produce fertile tetraploid offspring by self-pollinating or by mating with other tetraploids. In addition, the tetraploids are reproductively isolated from $2n$ plants of the original population, because the triploid ($3n$) offspring of such unions have reduced fertility. Thus, in just one generation, autopolyploidy can generate reproductive isolation without any geographic separation.

A second form of polyploidy can occur when two different species interbreed and produce hybrid offspring. Most such hybrids are sterile because the set of chromosomes from one species cannot pair during meiosis with the set of chromosomes from the other species. However, an infertile hybrid may be able to propagate itself asexually (as many plants can do). In subsequent generations, various mechanisms can change a sterile hybrid into a fertile polyploid called an **allopolyploid** (Figure 24.10). The allopolyploids are fertile when mating with each other but cannot interbreed with either parent species; thus, they represent a new biological species.

Although it can be challenging to study speciation in the field, scientists have documented at least five new plant species that have originated by polyploid speciation since 1850. One of these examples involves the origin of a new species of goatsbeard plant (genus *Tragopogon*) in the Pacific Northwest. *Tragopogon* first arrived in the region when humans introduced three European species in the early 1900s: *T. pratensis*, *T. dubius*, and *T. porrifolius*. These three species are now common weeds

▼ **Figure 24.9 Sympatric speciation by autopolyploidy.**



▼ **Figure 24.10 One mechanism for allopolyploid speciation in plants.** Most hybrids are sterile because their chromosomes are not homologous and cannot pair during meiosis. However, such a hybrid may be able to reproduce asexually. This diagram traces one mechanism that can produce fertile hybrids (allopolyploids) that are members of a new species. The new species has a diploid chromosome number equal to the sum of the diploid chromosome numbers of the two parent species.

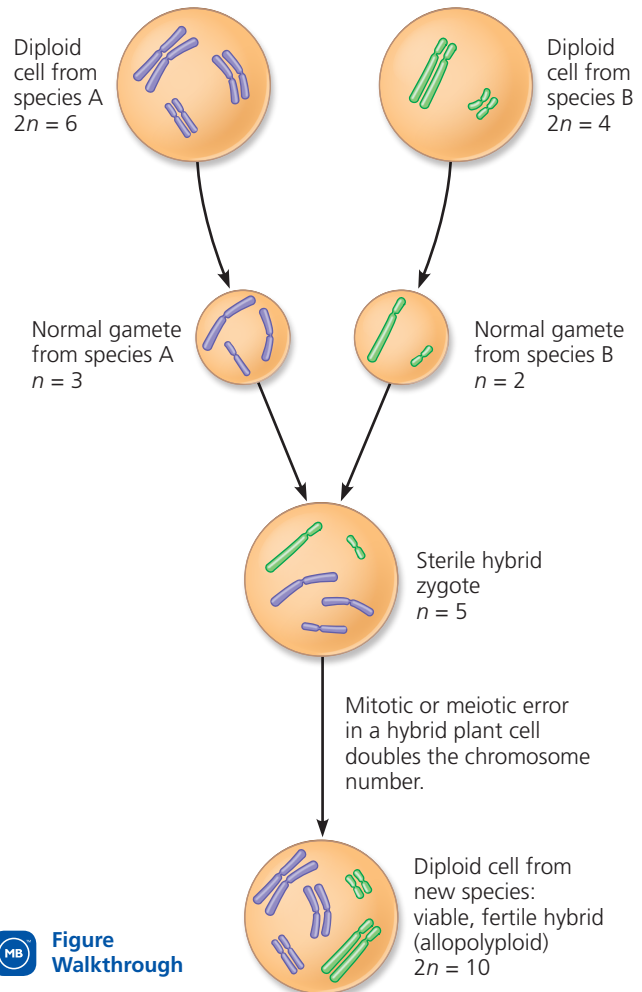


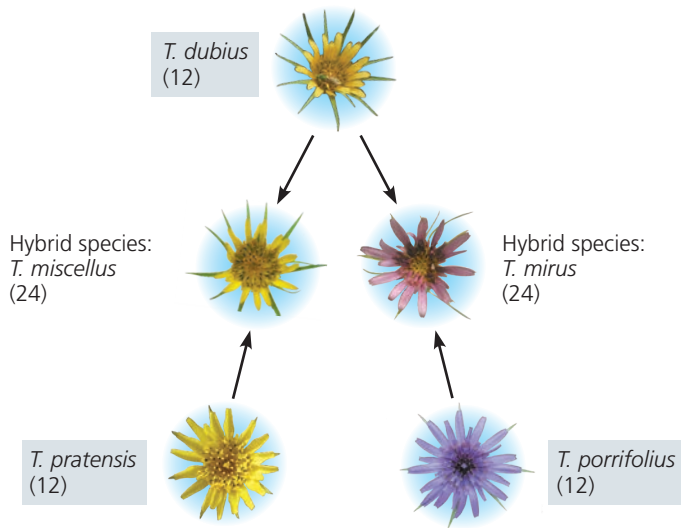
Figure Walkthrough

in abandoned parking lots and other urban sites. In 1950, a new *Tragopogon* species was discovered near the Idaho-Washington border, a region where all three European species also were found. Genetic analyses revealed that this new species, *Tragopogon miscellus*, is a hybrid of two of the European species (Figure 24.11). Although the *T. miscellus* population grows mainly by reproduction of its own members, additional episodes of hybridization between the parent species continue to add new members to the *T. miscellus* population. Later, scientists discovered another new *Tragopogon* species, *T. mirus*—this one a hybrid of *T. dubius* and *T. porrifolius* (see Figure 24.11). The *Tragopogon* story is just one of several well-studied examples in which scientists have observed speciation in progress.

Many important agricultural crops—such as oats, cotton, potatoes, tobacco, and wheat—are polyploids. For example, the wheat used for bread, *Triticum aestivum*, is an allohexaploid (six sets of chromosomes, two sets from each of three different species). The first of the polyploidy events that eventually led

▼ **Figure 24.11 Allopolyploid speciation in *Tragopogon*.**

The gray boxes indicate the three parent species. The diploid chromosome number of each species is shown in parentheses.



to modern wheat probably occurred about 8,000 years ago in the Middle East as a spontaneous hybrid of an early cultivated wheat species and a wild grass. Today, plant geneticists generate new polyploids in the laboratory by using chemicals that induce meiotic and mitotic errors. By harnessing the evolutionary process, researchers can produce new hybrid species with desired qualities, such as a hybrid that combines the high yield of wheat with the hardiness of rye.



Animation: Speciation by Changes in Ploidy

Sexual Selection

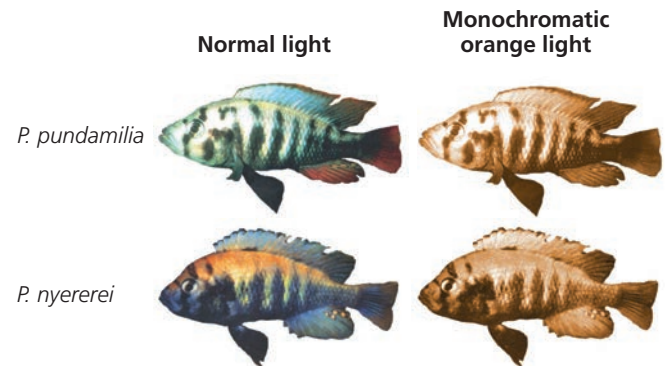
There is evidence that sympatric speciation can also be driven by sexual selection. Clues to how this can occur have been found in cichlid fish from one of Earth's hot spots of animal speciation, East Africa's Lake Victoria. This lake was once home to as many as 600 species of cichlids. Genetic data indicate that these species originated within the last 100,000 years from a small number of colonizing species that arrived from other lakes and rivers. How did so many species—more than double the number of freshwater fish species known in all of Europe—originate within a single lake?

One hypothesis is that subgroups of the original cichlid populations adapted to different food sources and the resulting genetic divergence contributed to speciation in Lake Victoria. But sexual selection, in which (typically) females select males based on their appearance (see Concept 23.4), may also have been a factor. Researchers have studied two closely related sympatric species of cichlids that differ mainly in the coloration of breeding males: Breeding *Pundamilia pundamilia* males have a blue-tinged back, whereas breeding *Pundamilia nyererei* males have a red-tinged back (**Figure 24.12**). Their results suggest that mate choice based on male breeding coloration can act as a reproductive barrier that keeps the gene pools of these two species separate.

▼ **Figure 24.12**

Inquiry Does sexual selection in cichlids result in reproductive isolation?

Experiment Researchers placed males and females of *Pundamilia pundamilia* and *P. nyererei* together in two aquarium tanks, one with natural light and one with a monochromatic orange lamp. Under normal light, the two species are noticeably different in male breeding coloration; under monochromatic orange light, the two species are very similar in color. The researchers then observed the mate choices of the females in each tank.



Results Under normal light, females of each species strongly preferred males of their own species. But under orange light, females of each species responded indiscriminately to males of both species. The resulting hybrids were viable and fertile.

Conclusion The researchers concluded that mate choice by females based on male breeding coloration can act as a reproductive barrier that keeps the gene pools of these two species separate. Since the species can still interbreed when this prezygotic behavioral barrier is breached in the laboratory, the genetic divergence between the species is likely to be small. This suggests that speciation in nature has occurred relatively recently.

Data from O. Seehausen and J. J. M. van Alphen, The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex), *Behavioral Ecology and Sociobiology* 42:1–8 (1998).

WHAT IF? > Suppose that female cichlids living in the murky waters of a polluted lake could not distinguish colors well. In such waters, how might the gene pools of these species change over time?

Habitat Differentiation

Sympatric speciation can also occur when a subpopulation exploits a habitat or resource not used by the parent population. Consider the North American apple maggot fly (*Rhagoletis pomonella*), a pest of apples. The fly's original habitat was the native hawthorn tree (see Figure 24.3a), but about 200 years ago, some populations colonized apple trees that had been introduced by European settlers. Apple maggot flies usually mate on or near their host plant. This results in a prezygotic barrier (habitat isolation) between populations that feed on apples and populations that feed on hawthorns. Furthermore, because apples mature more quickly than hawthorn fruit, natural selection has favored apple-feeding flies with rapid development. These apple-feeding populations now show temporal isolation from the hawthorn-feeding *R. pomonella*, providing a second prezygotic barrier to gene flow between

the two populations. Researchers also have identified alleles that benefit the flies that use one host plant but harm the flies that use the other host plant. Natural selection operating on these alleles has provided a postzygotic barrier to reproduction, further limiting gene flow. Altogether, although the two populations are still classified as subspecies rather than separate species, sympatric speciation appears to be well under way.

Allopatric and Sympatric Speciation: A Review

Now let's recap the processes by which new species form. In allopatric speciation, a new species forms in geographic isolation from its parent population. Geographic isolation severely restricts gene flow. Intrinsic barriers to reproduction with members of the parent population may then arise as a by-product of genetic changes that occur within the isolated population. Many different processes can produce such genetic changes, including natural selection under different environmental conditions, genetic drift, and sexual selection. Once formed, reproductive barriers that arise in allopatric populations can prevent interbreeding with the parent population even if the populations come back into contact.

Sympatric speciation, in contrast, requires the emergence of a reproductive barrier that isolates a subset of a population from the remainder of the population in the same area. Though rarer than allopatric speciation, sympatric speciation can occur when gene flow to and from the isolated subpopulation is blocked. This can occur as a result of polyploidy, a condition in which an organism has extra sets of chromosomes. Sympatric speciation also can result from sexual selection. Finally, sympatric speciation can occur when a subset of a population becomes reproductively isolated because of natural selection that results from a switch to a habitat or food source not used by the parent population.

Having reviewed the geographic context in which species originate, we'll next explore in more detail what can happen when new or partially formed species come into contact.



HHMI Video: Anole Lizards: An Example of Speciation



CONCEPT CHECK 24.2

1. Summarize key differences between allopatric and sympatric speciation. Which type of speciation is more common, and why?
2. Describe two mechanisms that can decrease gene flow in sympatric populations, thereby making sympatric speciation more likely to occur.
3. **WHAT IF? >** Is allopatric speciation more likely to occur on an island close to a mainland or on a more isolated island of the same size? Explain your prediction.
4. **MAKE CONNECTIONS >** Review the process of meiosis in Figure 13.8. Describe how an error during meiosis could lead to polyploidy.

For suggested answers, see Appendix A.

CONCEPT 24.3

Hybrid zones reveal factors that cause reproductive isolation

What happens if species with incomplete reproductive barriers come into contact with one another? One possible outcome is the formation of a **hybrid zone**, a region in which members of different species meet and mate, producing at least some offspring of mixed ancestry. In this section, we'll explore hybrid zones and what they reveal about factors that cause the evolution of reproductive isolation.

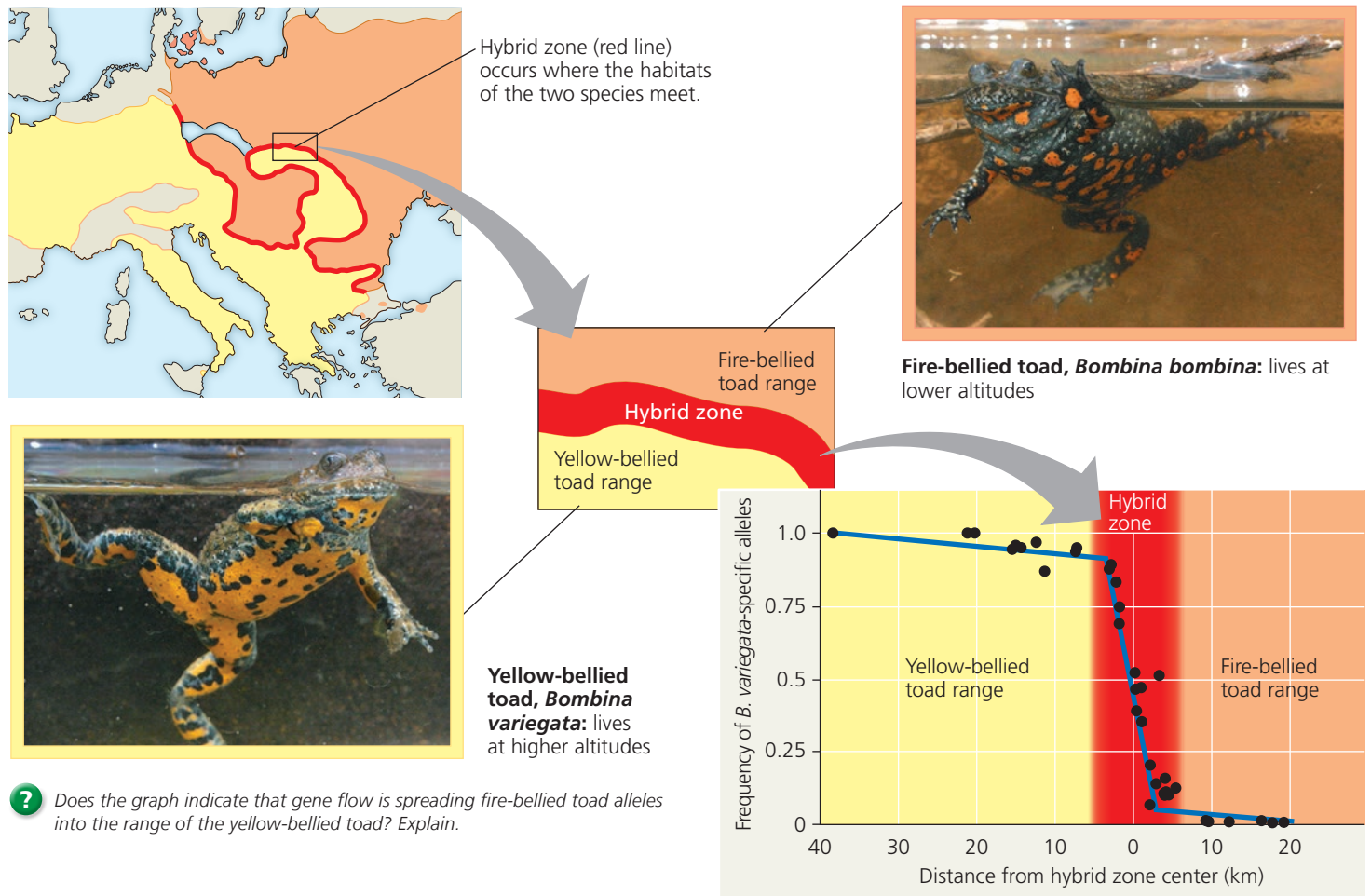
Patterns Within Hybrid Zones

Some hybrid zones form as narrow bands, such as the one depicted in **Figure 24.13** for the yellow-bellied toad (*Bombina variegata*) and its close relative, the fire-bellied toad (*B. bombina*). This hybrid zone, represented by the red line on the map, extends for 4,000 km but is less than 10 km wide in most places. The hybrid zone occurs where the higher-altitude habitat of the yellow-bellied toad meets the lowland habitat of the fire-bellied toad. Across a given "slice" of the zone, the frequency of alleles specific to yellow-bellied toads typically decreases from close to 100% at the edge where only yellow-bellied toads are found to around 50% in the central portion of the zone to close to 0% at the edge where only fire-bellied toads are found.

What causes such a pattern of allele frequencies across a hybrid zone? We can infer that there is an obstacle to gene flow—otherwise, alleles from one parent species would also be common in the gene pool of the other parent species. Are geographic barriers reducing gene flow? Not in this case, since the toads can move throughout the hybrid zone. A more important factor is that hybrid toads have increased rates of embryonic mortality and a variety of morphological abnormalities, including ribs that are fused to the spine and malformed tadpole mouthparts. Because the hybrids have poor survival and reproduction, they produce few viable offspring with members of the parent species. As a result, hybrid individuals rarely serve as a stepping-stone from which alleles are passed from one species to the other. Outside the hybrid zone, additional obstacles to gene flow may be provided by natural selection in the different environments in which the parent species live.

Hybrid zones typically are located wherever the habitats of the interbreeding species meet. Those regions often resemble a group of isolated patches scattered across the landscape—more like the complex pattern of spots on a Dalmatian than the continuous band shown in Figure 24.13. But regardless of whether they have complex or simple spatial patterns, hybrid zones form when two species lacking complete barriers to reproduction come into contact. What happens when the habitats of the interbreeding species change over time?

▼ **Figure 24.13 A narrow hybrid zone for *Bombina* toads in Europe.** The graph shows species-specific allele frequencies across the width of the zone near Krakow, Poland, averaged over six genetic loci. A value of 1.0 indicates that all individuals were yellow-bellied toads, 0 indicates that all individuals were fire-bellied toads, and intermediate frequencies indicate that some individuals were of mixed ancestry.



? Does the graph indicate that gene flow is spreading fire-bellied toad alleles into the range of the yellow-bellied toad? Explain.

Hybrid Zones and Environmental Change

A change in environmental conditions can alter where the habitats of interbreeding species meet. When this happens, an existing hybrid zone can move to a new location, or a novel hybrid zone may form.

For example, black-capped chickadees (*Parus atricapillus*) and Carolina chickadees (*P. carolinensis*) interbreed in a narrow hybrid zone that runs from New Jersey to Kansas. Recent studies have shown that the location of this hybrid zone has shifted northward as the climate has warmed. In another example, a series of warm winters prior to 2003 enabled the southern flying squirrel (*Glaucomys volans*) to expand northward into the range of the northern flying squirrel, *G. sabrinus*. Previously, the ranges of these two species had not overlapped. Genetic analyses showed that these flying squirrels began to hybridize where their ranges came into contact, thereby forming a novel hybrid zone induced by climate change.

Finally, note that a hybrid zone can be a source of novel genetic variation that improves the ability of one or both

parent species to cope with changing environmental conditions. This can occur when an allele found only in one parent species is transferred first to hybrid individuals, and then to the other parent species when hybrids breed with the second parent species. Recent genetic analyses have shown that hybridization has been a source for such novel genetic variation in various insect, bird, and plant species. In the **Problem-Solving Exercise**, you can examine one such example: a case in which hybridization may have led to the transfer of insecticide-resistance alleles between mosquitoes that transmit malaria.

Hybrid Zones over Time

Studying a hybrid zone is like observing a naturally occurring experiment on speciation. Will the hybrids become reproductively isolated from their parents and form a new species, as occurred by polyploidy in the goatsbeard plant of the Pacific Northwest? If not, there are three other common outcomes for the hybrid zone over time: reinforcement of

PROBLEM-SOLVING EXERCISE

Is hybridization promoting insecticide resistance in mosquitoes that transmit malaria?

Malaria is a leading cause of human illness and mortality worldwide, with 200 million people infected and 600,000 deaths each year. In the 1960s, the incidence of malaria was reduced owing to the use of insecticides that killed mosquitoes in the genus *Anopheles*, which transmit the disease from person to person. But today, mosquitoes are becoming resistant to insecticides—causing a resurgence in malaria.



▲ Insecticide-treated bed nets have helped reduce cases of malaria in many countries, but resistance to insecticides is rising in mosquito populations.



Instructors: A version of this Problem-Solving Exercise can be assigned in MasteringBiology.

In this exercise, you will investigate whether alleles encoding resistance to insecticides have been transferred between closely related species of *Anopheles*.

Your Approach The principle guiding your investigation is that DNA analyses can detect the transfer of resistance alleles between closely related mosquito species. To find out whether such transfers have occurred, you will analyze DNA results from two species of mosquitoes that transmit malaria (*Anopheles gambiae* and *A. coluzzii*) and from *A. gambiae* × *A. coluzzii* hybrids.

Your Data

Resistance to DDT and other insecticides in *Anopheles* is affected by a sodium channel gene, *kdr*. The *r* allele of this gene confers resistance, while the wild type (+/+) genotype is not resistant. Researchers sequenced the *kdr* gene from mosquitoes collected in Mali during three time periods: pre-2006 (2002 and 2004), 2006, and post-2006 (2009–2012). *A. gambiae* and *A. coluzzii* were collected during all three time periods, but their hybrids only occurred in 2006, the first year that insecticide-treated bed nets were used to reduce the spread of malaria. A likely explanation is that the introduction of the treated bed nets may have briefly favored hybrid individuals, which are usually at a selective disadvantage.

Observed numbers of mosquitoes by <i>kdr</i> genotype			
	+/+	+/r	r/r
<i>A. gambiae</i>			
Pre-2006	3	5	2
2006	8	8	7
Post-2006	3	3	57
Hybrids			
2006	10	7	0
<i>A. coluzzii</i>			
Pre-2006	226	0	0
2006	70	7	0
Post-2006	79	127	94

Your Analysis

1. How did the frequencies of *kdr* genotypes change over time in *A. gambiae*? Describe a hypothesis that accounts for these observations.
2. How did the frequencies of *kdr* genotypes change over time in *A. coluzzii*? Describe a hypothesis that accounts for these observations.
3. Do these results indicate that hybridization can lead to the transfer of adaptive alleles? Explain.
4. Predict how the transfer of the *r* allele to *A. coluzzii* populations could affect the number of malaria cases.

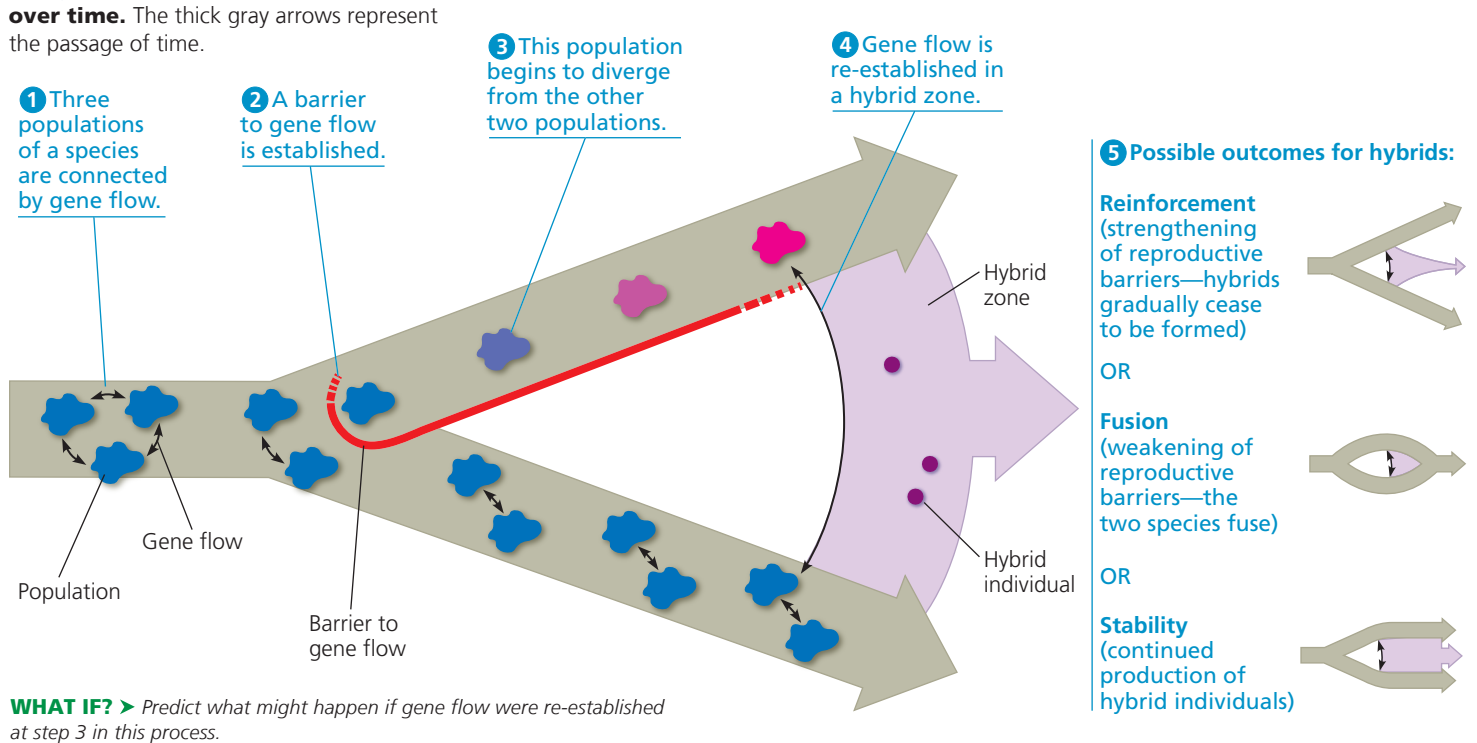
barriers, fusion of species, or stability (**Figure 24.14**). Let's examine what studies suggest about these possibilities.

Reinforcement: Strengthening Reproductive Barriers

Hybrids often are less fit than members of their parent species. In such cases, natural selection should strengthen prezygotic barriers to reproduction, reducing the formation of unfit hybrids. Because this process involves *reinforcing* reproductive barriers, it is called **reinforcement**. If reinforcement is occurring, a logical prediction is that barriers to reproduction between species should be stronger for sympatric populations than for allopatric populations.

As an example, let's consider two species of European flycatcher, the pied flycatcher (*Ficedula hypoleuca*) and the collared flycatcher (*Ficedula albicollis*). In allopatric populations of these birds, males of the two species closely resemble one another, while in sympatric populations, the males look very different. Female flycatchers do not select males of the other species when given a choice between males from sympatric populations, but they frequently do make mistakes when selecting between males from allopatric populations. Thus, barriers to reproduction are stronger in birds from sympatric populations than in birds from allopatric populations, as you would predict if reinforcement were occurring. Similar results have been observed in a number of organisms, including fishes, insects, plants, and other birds.

▼ **Figure 24.14 Formation of a hybrid zone and possible outcomes for hybrids over time.** The thick gray arrows represent the passage of time.



Fusion: Weakening Reproductive Barriers

Barriers to reproduction may be weak when two species meet in a hybrid zone. Indeed, so much gene flow may occur that reproductive barriers weaken further and the gene pools of the two species become increasingly alike. In effect, the speciation process reverses, eventually causing the two hybridizing species to fuse into a single species.

For example, genetic and morphological evidence indicate that the recent loss of the large tree finch from the Galápagos island of Floreana resulted from extensive hybridization with another finch species on that island. Such a situation also may be occurring among Lake Victoria cichlids. Many pairs of ecologically similar cichlid species are reproductively isolated because the females of one species prefer to mate with males of one color, while females of the other species prefer to mate with males of a different color (see Figure 24.12). Results from field and laboratory studies indicate that murky waters caused by pollution have reduced the ability of females to use color to distinguish males of their own species from males of closely related species. In some polluted waters, many hybrids have been produced, leading to fusion of the parent species' gene pools and a loss of species (**Figure 24.15**).

Stability: Continued Formation of Hybrid Individuals

Many hybrid zones are stable in the sense that hybrids continue to be produced. In some cases, this occurs because the hybrids survive or reproduce better than members of either parent species, at least in certain habitats or years. But stable

▼ **Figure 24.15 Fusion: the breakdown of reproductive barriers.**

Increasingly cloudy water in Lake Victoria over the past several decades may have weakened reproductive barriers between *P. nyererei* and *P. pundamilia*. In areas of cloudy water, the two species have hybridized extensively, causing their gene pools to fuse.



Pundamilia nyererei

Pundamilia pundamilia



Pundamilia "turbid water," hybrid offspring from a location with turbid water

hybrid zones have also been observed in cases where the hybrids are selected *against*—an unexpected result.

For example, hybrids continue to form in the *Bombina* hybrid zone even though they are strongly selected against.

One explanation relates to the narrowness of the *Bombina* hybrid zone (see Figure 24.13). Evidence suggests that members of both parent species migrate into the zone from the parent populations located outside the zone, thus leading to the continued production of hybrids. If the hybrid zone were wider, this would be less likely to occur, since the center of the zone would receive little gene flow from distant parent populations located outside the hybrid zone.

Sometimes the outcomes in hybrid zones match our predictions (European flycatchers and cichlid fishes), and sometimes they don't (*Bombina*). But whether our predictions are upheld or not, events in hybrid zones can shed light on how barriers to reproduction between closely related species change over time. In the next section, we'll examine how interactions between hybridizing species can also provide a glimpse into the speed and genetic control of speciation.

CONCEPT CHECK 24.3

1. What are hybrid zones, and why can they be viewed as "natural laboratories" in which to study speciation?
2. **WHAT IF? >** Consider two species that diverged while geographically separated but resumed contact before reproductive isolation was complete. Predict the outcome over time if the two species mated indiscriminately and (a) hybrid offspring survived and reproduced more poorly than offspring from intraspecific matings or (b) hybrid offspring survived and reproduced as well as offspring from intraspecific matings.

For suggested answers, see Appendix A.

CONCEPT 24.4

Speciation can occur rapidly or slowly and can result from changes in few or many genes

Darwin faced many questions when he began to ponder that "mystery of mysteries"—speciation. He found answers to some of those questions when he realized that evolution by natural selection helps explain both the diversity of life and the adaptations of organisms (see Concept 21.2). But biologists since Darwin have continued to ask fundamental questions about speciation. How long does it take for new species to form? And how many genes change when one species splits into two? Answers to these questions are also emerging.

The Time Course of Speciation

We can gather information about how long it takes new species to form from broad patterns in the fossil record and from studies that use morphological data (including fossils) or molecular data to assess the time interval between speciation events in particular groups of organisms.

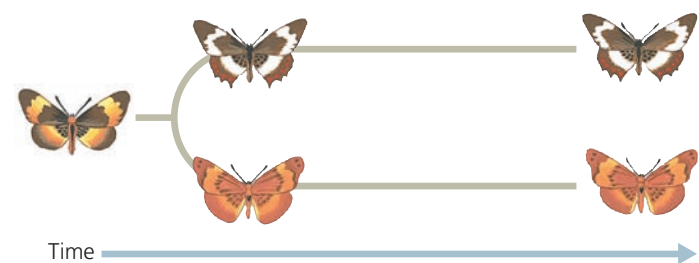
Patterns in the Fossil Record

The fossil record includes many episodes in which new species appear suddenly in a geologic stratum, persist essentially unchanged through several strata, and then disappear. For example, there are dozens of species of marine invertebrates that make their debut in the fossil record with novel morphologies, but then change little for millions of years before becoming extinct. The term **punctuated equilibria** is used to describe these periods of apparent stasis punctuated by sudden change (**Figure 24.16a**). Other species do not show a punctuated pattern; instead, they appear to have changed more gradually over long periods of time (**Figure 24.16b**).

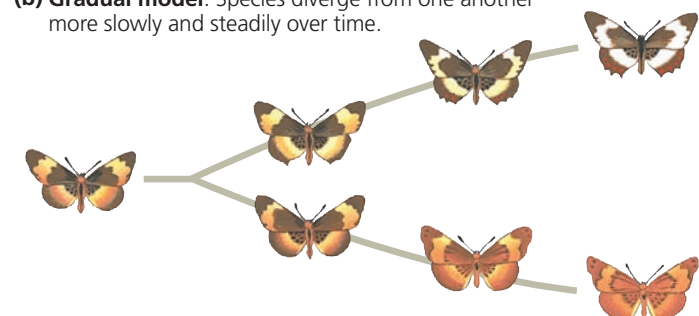
What might punctuated and gradual patterns tell us about how long it takes new species to form? Suppose that a species survived for 5 million years, but most of the morphological changes that caused it to be designated a new species occurred during the first 50,000 years of its existence—just 1% of its total lifetime. Time periods this short (in geologic terms) often cannot be distinguished in fossil strata, in part because the rate of sediment accumulation may be too slow to separate layers this close in time. Thus, based on its fossils, the species would seem to have appeared suddenly and then lingered with little or no change before becoming extinct. Even though such a species may have originated more slowly than its fossils suggest (in this case taking up to 50,000 years), a punctuated pattern indicates that speciation occurred relatively rapidly. For species whose fossils changed much more gradually, we also cannot tell exactly when a new biological species formed, since information about reproductive isolation does not fossilize.

▼ Figure 24.16 Two models for the tempo of speciation.

(a) **Punctuated model.** New species change most as they branch from a parent species and then change little for the rest of their existence.



(b) **Gradual model.** Species diverge from one another more slowly and steadily over time.



However, it is likely that speciation in such groups occurred relatively slowly, perhaps taking millions of years.



Interview with Stephen Jay Gould: An “architect” of the concept of punctuated equilibria

Speciation Rates

The existence of fossils that display a punctuated pattern suggests that once the process of speciation begins, it can be completed relatively rapidly—a suggestion supported by a growing number of studies.

For example, rapid speciation appears to have produced the wild sunflower *Helianthus anomalus*. Genetic evidence indicates that this species originated by the hybridization of two other sunflower species, *H. annuus* and *H. petiolaris*. The hybrid species *H. anomalus* is ecologically distinct and reproductively isolated from both parent species (Figure 24.17). Unlike the outcome of allopolyploid speciation, in which there is a change in chromosome number after hybridization, in these sunflowers the two parent species and the hybrid all have the same number of chromosomes ($2n = 34$). How, then, did speciation occur? To study this question, researchers performed an experiment designed to mimic events in nature (Figure 24.18). Their results indicated that natural selection could produce extensive genetic changes in hybrid populations over short periods of time. These changes appear to have caused the hybrids to diverge reproductively from their parents and form a new species, *H. anomalus*.

The sunflower example, along with the apple maggot fly, Lake Victoria cichlid, and fruit fly examples discussed earlier, suggests that new species can arise rapidly *once divergence begins*. But what is the total length of time between speciation events? This interval consists of the time that elapses before populations of a newly formed species start to diverge from one another plus the time it takes for speciation to be complete once divergence begins. It turns out that the total time between speciation events varies considerably. In a survey of data from 84 groups of plants and animals, speciation intervals ranged from 4,000 years (in cichlids of Lake Nabugabo,

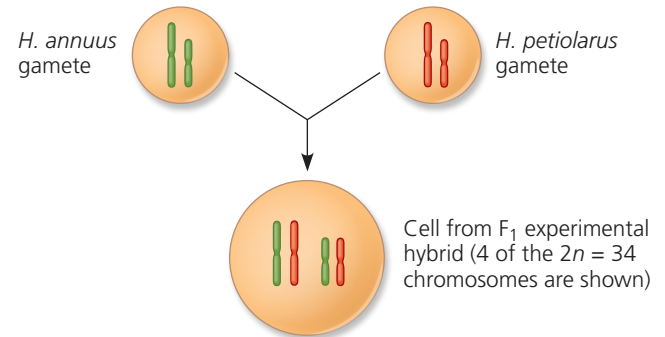
Figure 24.17 A hybrid sunflower species and its dry sand dune habitat. The wild sunflower *Helianthus anomalus* shown here originated via the hybridization of two other sunflowers, *H. annuus* and *H. petiolaris*, which live in nearby but moister environments.



Figure 24.18

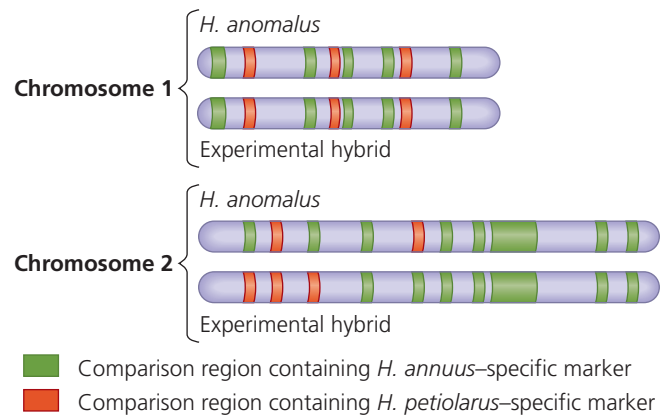
Inquiry How does hybridization lead to speciation in sunflowers?

Experiment Loren Rieseberg and his colleagues crossed the two parent sunflower species, *H. annuus* and *H. petiolaris*, to produce experimental hybrids in the laboratory (for each gamete, only two of the $n = 17$ chromosomes are shown).



Note that in the first (F_1) generation, each chromosome of the experimental hybrids consisted entirely of DNA from one or the other parent species. The researchers then tested whether the F_1 and subsequent generations of experimental hybrids were fertile. They also used species-specific genetic markers to compare the chromosomes in the experimental hybrids with the chromosomes in the naturally occurring hybrid *H. anomalus*.

Results Although only 5% of the F_1 experimental hybrids were fertile, after just four more generations the hybrid fertility rose to more than 90%. The chromosomes of individuals from this fifth hybrid generation differed from those in the F_1 generation (see above) but were similar to those in *H. anomalus* individuals from natural populations:



Conclusion Over time, the chromosomes in the population of experimental hybrids became similar to the chromosomes of *H. anomalus* individuals from natural populations. This suggests that the observed rise in the fertility of the experimental hybrids may have occurred as selection eliminated regions of DNA from the parent species that were not compatible with one another. Overall, it appeared that the initial steps of the speciation process occurred rapidly and could be mimicked in a laboratory experiment.

Data from L. H. Rieseberg et al., Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids, *Science* 272:741–745 (1996).

WHAT IF? The increased fertility of the experimental hybrids could have resulted from natural selection for thriving under laboratory conditions. Evaluate this alternative explanation for the result.

Uganda) to 40 million years (in some beetles). Overall, the time between speciation events averaged 6.5 million years and was rarely less than 500,000 years.

These data suggest that on average, millions of years may pass before a newly formed plant or animal species will itself give rise to another new species. As you'll read in Concept 25.4, this finding has implications for how long it takes life on Earth to recover from mass extinction events. Moreover, the extreme variability in the time it takes new species to form indicates that organisms do not have an internal "speciation clock" that causes them to produce new species at regular intervals. Instead, speciation begins only after gene flow between populations is interrupted, perhaps by changing environmental conditions or by unpredictable events, such as a storm that transports a few individuals to a new area. Furthermore, once gene flow is interrupted, the populations must diverge genetically to such an extent that they become reproductively isolated—all before other events cause gene flow to resume, possibly reversing the speciation process (see Figure 24.15).

Studying the Genetics of Speciation

Studies of ongoing speciation (as in hybrid zones) can reveal traits that cause reproductive isolation. By identifying the genes that control those traits, scientists can explore a fundamental question of evolutionary biology: How many genes influence the formation of new species?

In some cases, the evolution of reproductive isolation results from the effects of a single gene. For example, in Japanese snails of the genus *Euhadra*, a change in a single gene results in a mechanical barrier to reproduction. This gene controls the direction in which the shells spiral. When their shells spiral in different directions, the snails' genitalia are oriented in a manner that prevents mating (Figure 24.3f shows a similar example). Recent genetic analyses have uncovered other single genes that cause reproductive isolation in fruit flies or mice.

A major barrier to reproduction between two closely related species of monkey flower, *Mimulus cardinalis* and *M. lewisii*, also appears to be influenced by a relatively small number of genes. These two species are isolated by several prezygotic and postzygotic barriers. Of these, one prezygotic barrier, pollinator choice, accounts for most of the isolation: In a hybrid zone between *M. cardinalis* and *M. lewisii*, nearly 98% of pollinator visits were restricted to one species or the other.

The two monkey flower species are visited by different pollinators: Hummingbirds prefer the red-flowered *M. cardinalis*, and bumblebees prefer the pink-flowered *M. lewisii*. Pollinator choice is affected by at least two loci in the monkey flowers, one of which, the "yellow upper," or *yup*, locus, influences flower color (Figure 24.19). By crossing the two parent species to produce F₁ hybrids and then performing repeated backcrosses of these F₁ hybrids to each parent species, researchers succeeded in transferring the *M. cardinalis* allele at this locus into *M. lewisii*, and vice versa. In a field experiment, *M. lewisii*

▼ Figure 24.19 A locus that influences pollinator choice.

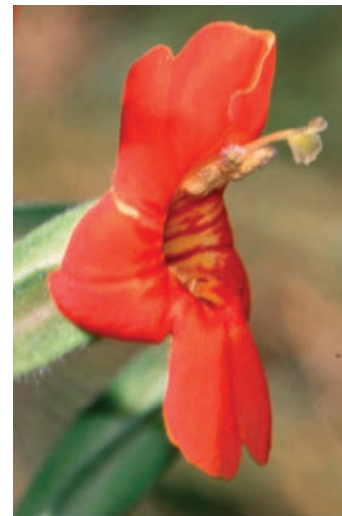
Pollinator preferences provide a strong barrier to reproduction between *Mimulus lewisii* and *M. cardinalis*. After transferring the *M. lewisii* allele for a flower-color locus into *M. cardinalis* and vice versa, researchers observed a shift in some pollinators' preferences.



(a) Typical *Mimulus lewisii*



(b) *M. lewisii* with an *M. cardinalis* flower-color allele



(c) Typical *Mimulus cardinalis*



(d) *M. cardinalis* with an *M. lewisii* flower-color allele

WHAT IF ► If *M. cardinalis* individuals that had the *M. lewisii* *yup* allele were planted in an area that housed both monkey flower species, how might the production of hybrid offspring be affected?

plants with the *M. cardinalis* *yup* allele received 68-fold more visits from hummingbirds than did wild-type *M. lewisii*. Similarly, *M. cardinalis* plants with the *M. lewisii* *yup* allele received 74-fold more visits from bumblebees than did wild-type *M. cardinalis*. Thus, a mutation at a single locus can influence pollinator preference and hence contribute to reproductive isolation in monkey flowers.

In other organisms, the speciation process is influenced by larger numbers of genes and gene interactions. For example, hybrid sterility between two subspecies of the fruit fly *Drosophila pseudoobscura* results from gene interactions among at least four loci, and postzygotic isolation in the sunflower hybrid zone discussed earlier is influenced by at least 26 chromosome segments (and an unknown number of genes). Overall, studies suggest that few or many genes can influence the evolution of reproductive isolation and hence the emergence of a new species.

From Speciation to Macroevolution

As you've seen, speciation may begin with differences as small as the color on a cichlid's back. However, as speciation occurs again and again, such differences can accumulate and become more pronounced, eventually leading to the formation of new groups of organisms that differ greatly from their ancestors (as in the origin of whales from terrestrial mammals; see Figure 21.20). Moreover, as one group of organisms increases in size by producing many new species, another group of organisms may shrink, losing species to extinction. The cumulative effects of many such speciation and extinction events have helped shape the sweeping evolutionary changes that are documented in the fossil record. In the next chapter, we turn to such

large-scale evolutionary changes as we begin our study of macroevolution.

CONCEPT CHECK 24.4

1. Speciation can occur rapidly between diverging populations, yet the time between speciation events is often more than a million years. Explain this apparent contradiction.
2. Summarize evidence that the *yup* locus acts as a prezygotic barrier to reproduction in two species of monkey flowers. Do these results demonstrate that the *yup* locus alone controls barriers to reproduction between these species? Explain.
3. **MAKE CONNECTIONS** > Compare Figure 13.12 with Figure 24.18. What cellular process could cause the hybrid chromosomes in Figure 24.18 to contain DNA from both parent species? Explain.

For suggested answers, see Appendix A.

24 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 24.1

The biological species concept emphasizes reproductive isolation (pp. 561–564)

- A biological **species** is a group of populations whose individuals may interbreed and produce viable, fertile offspring with each other but not with members of other species.
- The **biological species concept** emphasizes reproductive isolation through **prezygotic** and **postzygotic barriers** that separate gene pools.

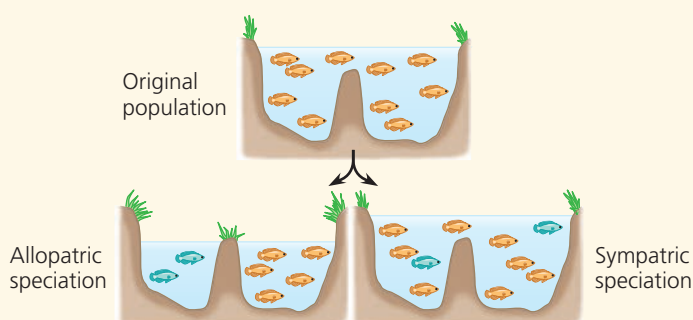
? Explain the role of gene flow in the biological species concept.



CONCEPT 24.2

Speciation can take place with or without geographic separation (pp. 565–570)

- In **allopatric speciation**, gene flow is reduced when two populations of one species become geographically separated from each other. One or both populations may undergo evolutionary change during the period of separation, resulting in the establishment of barriers to reproduction.



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- In **sympatric speciation**, a new species originates while remaining in the same geographic area as the parent species. Plant species (and, more rarely, animal species) have evolved sympatrically through **polyploidy**. Sympatric speciation can also result from sexual selection and habitat shifts.

? Can factors that cause sympatric speciation also cause allopatric speciation? Explain.

CONCEPT 24.3

Hybrid zones reveal factors that cause reproductive isolation (pp. 570–574)

- Many groups of organisms form **hybrid zones** in which members of different species meet and mate, producing at least some offspring of mixed ancestry.
- Many hybrid zones are **stable**, in that hybrid offspring continue to be produced over time. In others, **reinforcement** strengthens prezygotic barriers to reproduction, thus decreasing the formation of unfit hybrids. In still other hybrid zones, barriers to reproduction may weaken over time, resulting in the **fusion** of the species' gene pools (reversing the speciation process).

? What factors can support the long-term stability of a hybrid zone if the parent species live in different environments?

CONCEPT 24.4

Speciation can occur rapidly or slowly and can result from changes in few or many genes (pp. 574–577)

- New species can form rapidly once divergence begins—but it can take millions of years for that to happen. The time interval between speciation events varies considerably, from a few thousand years to tens of millions of years.
- Researchers have identified particular genes involved in some cases of speciation. Speciation can be driven by few or many genes.

? Is speciation something that happened only in the distant past, or are new species continuing to arise today? Explain.

Macroevolution

25

▲ **Figure 25.1** Would you have expected to find whale bones buried here?

KEY CONCEPTS

- 25.1** Conditions on early Earth made the origin of life possible
- 25.2** The fossil record documents the history of life
- 25.3** Key events in life's history include the origins of unicellular and multicellular organisms and the colonization of land
- 25.4** The rise and fall of groups of organisms reflect differences in speciation and extinction rates
- 25.5** Major changes in body form can result from changes in the sequences and regulation of developmental genes
- 25.6** Evolution is not goal oriented

▼ Fossil of *Dorudon atrox*, an ancient whale



A Surprise in the Desert

With its dry, wind-sculpted sands and searing heat, the Sahara Desert seems an unlikely place to discover the bones of whales. But starting in the 1870s, researchers uncovered fossils of ancient whales at several locations that once were covered by an ancient sea (**Figure 25.1**). For example, a nearly complete skeleton of *Dorudon atrox*, an extinct whale that lived 35 million years ago, was discovered in a region that came to be called Wadi Hitan, the “Valley of Whales.” Collectively, the whale fossils found in the Sahara were spectacular not only for where they were found, but also for documenting early steps in the transition from life on land to life in the sea.

Fossils discovered in other parts of the world tell a similar story: Past organisms were very different from those presently living. The sweeping changes in life on Earth as revealed by fossils illustrate **macroevolution**, the broad pattern of evolution above the species level. Examples of macroevolutionary change include the emergence of terrestrial vertebrates through a series of speciation events, the impact of mass extinctions on biodiversity, and the origin of key adaptations such as flight.

Taken together, such changes provide a grand view of the evolutionary history of life. We'll examine that history in this chapter, beginning with a discussion of hypotheses regarding the origin of life. This is the most speculative topic of the entire unit, for no fossil evidence of that seminal episode exists. We will then turn to evidence from the fossil record about major events in the history of life and the factors that have shaped the rise and fall of different groups of organisms over time.

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Get Ready for This Chapter

CONCEPT 25.1

Conditions on early Earth made the origin of life possible

Direct evidence of life on early Earth comes from fossils of microorganisms that lived 3.5 billion years ago. But how did the first living cells appear? Observations and experiments in chemistry, geology, and physics have led scientists to propose one scenario that we'll examine here. They hypothesize that chemical and physical processes could have produced simple cells through a sequence of four main stages:

1. The abiotic (nonliving) synthesis of small organic molecules, such as amino acids and nitrogenous bases
2. The joining of these small molecules into macromolecules, such as proteins and nucleic acids
3. The packaging of these molecules into **protocells**, droplets with membranes that maintained an internal chemistry different from that of their surroundings
4. The origin of self-replicating molecules that eventually made inheritance possible

Though speculative, this scenario leads to predictions that can be tested in the laboratory. In this section, we'll examine some of the evidence for each stage.

Synthesis of Organic Compounds on Early Earth

Our planet formed 4.6 billion years ago, condensing from a vast cloud of dust and rocks that surrounded the young sun. For its first few hundred million years, Earth was bombarded by huge chunks of rock and ice left over from the formation of the solar system. The collisions generated so much heat that all of the available water was vaporized, preventing the formation of seas and lakes.

This massive bombardment ended 4 billion years ago, setting the stage for the origin of life. The first atmosphere had little oxygen and was likely thick with water vapor, along with compounds released by volcanic eruptions, such as nitrogen and its oxides, carbon dioxide, methane, ammonia, and hydrogen. As Earth cooled, the water vapor condensed into oceans, and much of the hydrogen escaped into space.

During the 1920s, Russian chemist A. I. Oparin and British scientist J. B. S. Haldane independently hypothesized that Earth's early atmosphere was a reducing (electron-adding) environment, in which organic compounds could have formed from simpler molecules. The energy for this synthesis could have come from lightning and UV radiation. Haldane suggested that the early oceans were a solution of organic molecules, a "primitive soup" from which life arose.

In 1953, Stanley Miller and Harold Urey, working at the University of Chicago, tested the Oparin-Haldane hypothesis

▼ **Figure 25.2 Amino acid synthesis in a simulated volcanic eruption.** In addition to his classic 1953 study, Miller also conducted an experiment simulating a volcanic eruption. In a 2008 reanalysis of those results, researchers found that far more amino acids were produced under simulated volcanic conditions than were produced in the conditions of the original 1953 experiment.



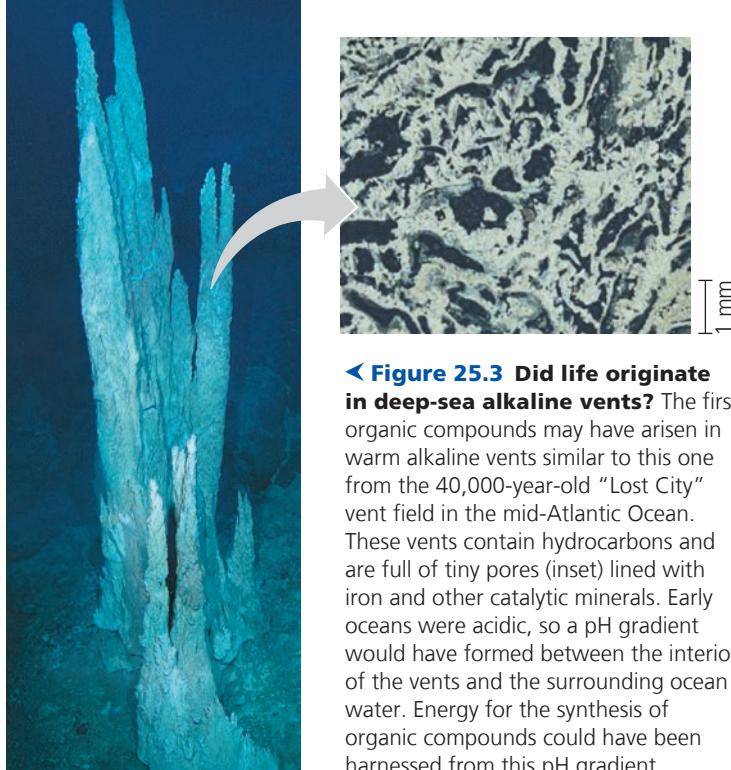
MAKE CONNECTIONS ► Explain how more than 20 amino acids could have been produced in the 2008 experiment. (See Concept 5.4.)

by creating laboratory conditions comparable to those that scientists at the time thought existed on early Earth (see Figure 4.2). His apparatus yielded a variety of amino acids found in organisms today, along with other organic compounds. Many laboratories have since repeated Miller's classic experiment using different recipes for the atmosphere, some of which also produced organic compounds.

However, some evidence suggests that the early atmosphere was made up primarily of nitrogen and carbon dioxide and was neither reducing nor oxidizing (electron removing). Recent Miller/Urey-type experiments using such "neutral" atmospheres have also produced organic molecules. In addition, small pockets of the early atmosphere, such as those near the openings of volcanoes, may have been reducing. Perhaps the first organic compounds formed near volcanoes. In 2008, researchers used modern equipment to reanalyze molecules that Miller had saved from one of his experiments. The 2008 paper found that numerous amino acids had formed under conditions that simulated a volcanic eruption (**Figure 25.2**).

Another hypothesis is that organic compounds were first produced in deep-sea **hydrothermal vents**, areas on the seafloor where heated water and minerals gush from Earth's interior into the ocean. Some of these vents, known as "black smokers," release water so hot (300–400°C) that organic compounds formed there may have been unstable. But other deep-sea vents, called **alkaline vents**, release water that has a high pH (9–11) and is warm (40–90°C) rather than hot, an environment that may have been more suitable for the origin of life (**Figure 25.3**).

Studies related to the volcanic-atmosphere and alkaline-vent hypotheses show that the abiotic synthesis of organic molecules is possible under various conditions. Another source of organic molecules may have been meteorites. For example, fragments of the Murchison meteorite, a 4.5-billion-year-old rock that landed in Australia in 1969, contain more than 80 amino acids, some in large amounts. These amino



◀ **Figure 25.3 Did life originate in deep-sea alkaline vents?** The first organic compounds may have arisen in warm alkaline vents similar to this one from the 40,000-year-old “Lost City” vent field in the mid-Atlantic Ocean. These vents contain hydrocarbons and are full of tiny pores (inset) lined with iron and other catalytic minerals. Early oceans were acidic, so a pH gradient would have formed between the interior of the vents and the surrounding ocean water. Energy for the synthesis of organic compounds could have been harnessed from this pH gradient.

acids cannot be contaminants from Earth because they consist of an equal mix of D and L isomers (see Figure 4.7). Organisms make and use only L isomers, with a few rare exceptions. Recent studies have shown that the Murchison meteorite also contained other key organic molecules, including lipids, simple sugars, and nitrogenous bases such as uracil.

Abiotic Synthesis of Macromolecules

The presence of small organic molecules, such as amino acids and nitrogenous bases, is not sufficient for the emergence of life as we know it. Every cell has many types of macromolecules, including enzymes and other proteins and the nucleic acids needed for self-replication. Could such macromolecules have formed on early Earth? A 2009 study demonstrated that one key step, the abiotic synthesis of RNA monomers, can occur spontaneously from simple precursor molecules. In addition, by dripping solutions of amino acids or RNA nucleotides onto hot sand, clay, or rock, researchers have produced polymers of these molecules. The polymers formed spontaneously, without the help of enzymes or ribosomes. Unlike proteins, the amino acid polymers are a complex mix of linked and cross-linked amino acids. Still, it is possible that such polymers acted as weak catalysts for a variety of chemical reactions on early Earth.

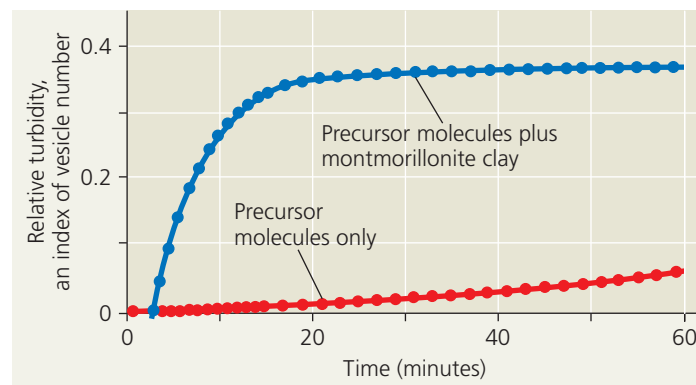
Protocells

All organisms must be able to carry out both reproduction and energy processing (metabolism). DNA molecules carry genetic information, including the instructions needed to replicate themselves accurately during reproduction. But DNA replication requires elaborate enzymatic machinery, along with an abundant supply of nucleotide building blocks provided by the cell’s metabolism. This suggests that self-replicating molecules

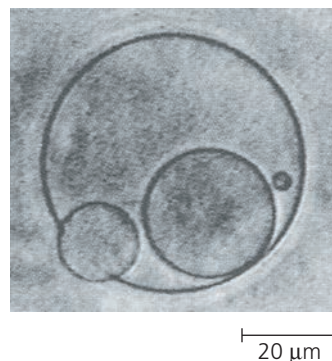
and a metabolic source of building blocks may have appeared together in early protocells. The necessary conditions may have been met in *vesicles*, fluid-filled compartments enclosed by a membrane-like structure. Recent experiments show that abiotically produced vesicles can exhibit certain properties of life, including simple reproduction and metabolism, as well as the maintenance of an internal chemical environment different from that of their surroundings (**Figure 25.4**).

For example, vesicles can form spontaneously when lipids or other organic molecules are added to water. When this occurs, molecules that have both a hydrophobic region and a hydrophilic region can organize into a bilayer similar to the lipid bilayer of a plasma membrane. Adding substances such as *montmorillonite*, a soft mineral clay produced by the weathering of volcanic ash, greatly increases the rate of vesicle self-assembly (see Figure 25.4a). This clay, which is thought to have been common on early Earth, provides surfaces on which organic molecules become concentrated, increasing the likelihood that the molecules will react with each other and form vesicles. Abiotically produced vesicles can “reproduce” on their own (see Figure 25.4b), and they can increase

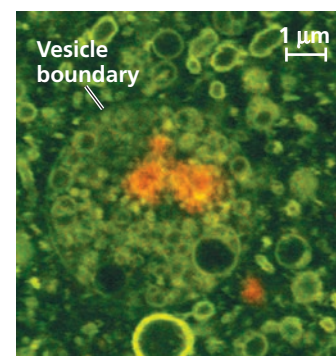
▼ **Figure 25.4 Features of abiotically produced vesicles.**



(a) **Self-assembly.** The presence of montmorillonite clay greatly increases the rate of vesicle self-assembly.



(b) **Reproduction.** Vesicles can divide on their own, as in this vesicle “giving birth” to smaller vesicles (LM).



(c) **Absorption of RNA.** This vesicle has incorporated montmorillonite clay particles coated with RNA (orange).

MAKE CONNECTIONS ► Explain how molecules with both a hydrophobic region and a hydrophilic region can self-assemble into a bilayer when in water. (See Concept 5.3.)

in size (“grow”) without dilution of their contents. Vesicles also can absorb montmorillonite particles, including those on which RNA and other organic molecules have become attached (see Figure 25.4c). Finally, experiments have shown that some vesicles have a selectively permeable bilayer and can perform metabolic reactions using an external source of reagents—another important prerequisite for life.

Self-Replicating RNA

The first genetic material was most likely RNA, not DNA. RNA plays a central role in protein synthesis, but it can also function as an enzyme-like catalyst (see Concept 17.3). Such RNA catalysts are called **ribozymes**. Some ribozymes can make complementary copies of short pieces of RNA, provided that they are supplied with nucleotide building blocks.

Natural selection on the molecular level has produced ribozymes capable of self-replication in the laboratory. How does this occur? Unlike double-stranded DNA, which takes the form of a uniform helix, single-stranded RNA molecules assume a variety of specific three-dimensional shapes mandated by their nucleotide sequences. In a given environment, RNA molecules with certain nucleotide sequences may have shapes that enable them to replicate faster and with fewer errors than other sequences. The RNA molecule with the greatest ability to replicate itself will leave the most descendant molecules. Occasionally, a copying error will result in a molecule with a shape that is even more adept at self-replication. Similar selection events may have occurred on early Earth. Thus, life as we know it may have been preceded by an “RNA world,” in which small RNA molecules were able to replicate and to store genetic information about the vesicles that carried them.

In 2013, Dr. Jack Szostak and colleagues succeeded in building a vesicle in which copying of a template strand of RNA could occur—a key step towards constructing a vesicle with self-replicating RNA. On early Earth, a vesicle with such self-replicating, catalytic RNA would differ from its many neighbors that lacked such molecules. If that vesicle could grow, split, and pass its RNA molecules to its “daughters,” the daughters would be protocells. Although the first such protocells likely carried only limited amounts of genetic information, specifying only a few properties, their inherited characteristics could have been acted on by natural selection. The most successful of the early protocells would have increased in number because they could exploit their resources effectively and pass their abilities on to subsequent generations.

Once RNA sequences that carried genetic information appeared in protocells, many additional changes would have been possible. For example, RNA could have provided the template on which DNA nucleotides were assembled. Double-stranded DNA is a more chemically stable repository for genetic information than is the more fragile RNA. DNA also can be replicated more accurately. Accurate replication was advantageous as genomes grew larger through gene

duplication and other processes and as more properties of the protocells became coded in genetic information. Once DNA appeared, the stage was set for a blossoming of new forms of life—a change we see documented in the fossil record.



Interview with Jack Szostak: Studying the origin of life

CONCEPT CHECK 25.1

1. What conditions on early Earth could have permitted the synthesis of organic molecules?
2. How would the appearance of protocells have represented a key step in the origin of life?
3. **MAKE CONNECTIONS** ▶ In changing from an “RNA world” to today’s “DNA world,” genetic information must have flowed from RNA to DNA. After reviewing Figures 17.4 and 26.9, suggest how this could have occurred. Does such a flow occur today?

For suggested answers, see Appendix A.

CONCEPT 25.2

The fossil record documents the history of life

Starting with the earliest traces of life, the fossil record opens a window into the world of long ago and provides glimpses of the evolution of life over billions of years. In this section, we’ll examine fossils as a form of scientific evidence: how fossils form, how scientists date and interpret them, and what they can and cannot tell us about changes in the history of life.

The Fossil Record

Sedimentary rocks are the richest source of fossils. As a result, the fossil record is based primarily on the sequence in which fossils have accumulated in sedimentary rock layers, called *strata* (see Figure 21.3). Useful information is also provided by other types of fossils, such as insects preserved in amber (fossilized tree sap) and mammals frozen in ice.

The fossil record shows that there have been great changes in the kinds of organisms on Earth at different points in time (**Figure 25.5**). Many past organisms were unlike organisms living today, and many organisms that once were common are now extinct. As we’ll see later in this section, fossils also document how new groups of organisms arose from previously existing ones.

As substantial and significant as the fossil record is, keep in mind that it is an incomplete chronicle of evolution. Many of Earth’s organisms did not die in the right place and time to be preserved as fossils. Of those fossils that were formed, many were destroyed by later geologic processes, and only a fraction of the others have been discovered. As a result, the known fossil record is biased in favor of species that existed for a long time, were abundant and widespread in certain kinds of environments, and had hard shells, skeletons, or other parts that

▼ **Figure 25.5 Documenting the history of life.** These fossils illustrate representative organisms from different points in time. Although prokaryotes and unicellular eukaryotes are shown only at the base of the diagram, these organisms continue to thrive today. In fact, most organisms on Earth are unicellular.

▼ *Dimetrodon*, the largest known carnivore of its day, was more closely related to mammals than to reptiles. The spectacular “sail” on its back may have functioned in temperature regulation or as an ornament that served to attract mates.



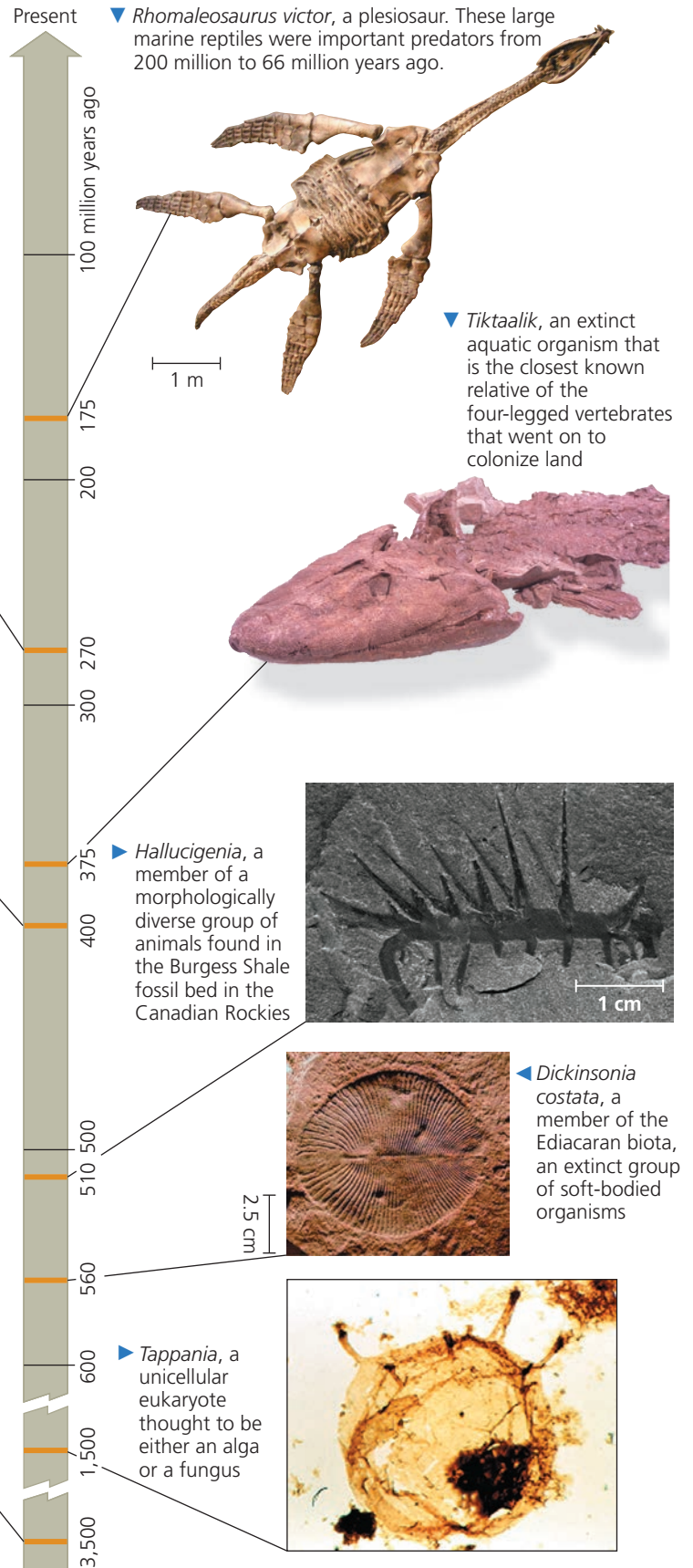
▲ *Coccosteus cuspidatus*, a placoderm (fishlike vertebrate) that had a bony shield covering its head and front end



▲ Some prokaryotes bind thin films of sediments together, producing layered rocks called stromatolites. Present-day stromatolites are found in a few shallow marine bays, such as Shark Bay, Australia, shown here.



▲ A section through a fossilized stromatolite



facilitated their fossilization. Even with its limitations, however, the fossil record is a remarkably detailed account of biological change over the vast scale of geologic time. Furthermore, as shown by the recently unearthed fossils of whale ancestors with hind limbs (see Figures 21.19, 21.20, and 25.1), gaps in the fossil record continue to be filled by new discoveries.

How Rocks and Fossils Are Dated

Fossils are valuable data for reconstructing the history of life, but only if we can determine where they fit in that unfolding story. While the order of fossils in rock strata tells us the sequence in which the fossils were laid down—their relative ages—it does not tell us their actual ages. Examining the relative positions of fossils is like peeling off layers of wallpaper in an old house. You can infer the sequence in which the layers were applied, but not the year each layer was added.

How can we determine the age of a fossil? One of the most common techniques is **radiometric dating**, which is based on the decay of radioactive isotopes (see Concept 2.2). In this process, a radioactive “parent” isotope decays to a “daughter” isotope at a characteristic rate. The rate of decay is expressed by the **half-life**, the time required for 50% of the parent isotope to decay (Figure 25.6). Each type of radioactive isotope has a characteristic half-life, which is not affected by temperature, pressure, or other environmental variables. For example, carbon-14 decays relatively quickly; its half-life is 5,730 years. Uranium-238 decays slowly; its half-life is 4.5 billion years.

Fossils contain isotopes of elements that accumulated in the organisms when they were alive. For example, a living organism contains the most common carbon isotope, carbon-12, as well as a radioactive isotope, carbon-14. When the organism dies, it stops accumulating carbon, and the amount of carbon-12 in its tissues does not change over time. However, the carbon-14 that it contains at the time of death slowly decays into another element, nitrogen-14. Thus, by measuring the ratio of carbon-14 to carbon-12 in a fossil, we can determine the fossil’s age. This method works for fossils up to about 75,000 years old; fossils

older than that contain too little carbon-14 to be detected with current techniques. Radioactive isotopes with longer half-lives are used to date older fossils.

Determining the age of these older fossils in sedimentary rocks can be challenging. Organisms do not use radioisotopes with long half-lives, such as uranium-238, to build their bones or shells. In addition, sedimentary rocks are often composed of sediments of differing ages. Although we cannot date these older fossils directly, an indirect method can be used to infer the age of fossils that are sandwiched between two layers of volcanic rock. As lava cools into volcanic rock, radioisotopes from the surrounding environment become trapped in the newly formed rock. Some of the trapped radioisotopes have long half-lives, allowing geologists to estimate the ages of ancient volcanic rocks. If two volcanic layers surrounding fossils are found to be 525 million and 535 million years old, for example, then the fossils are roughly 530 million years old.

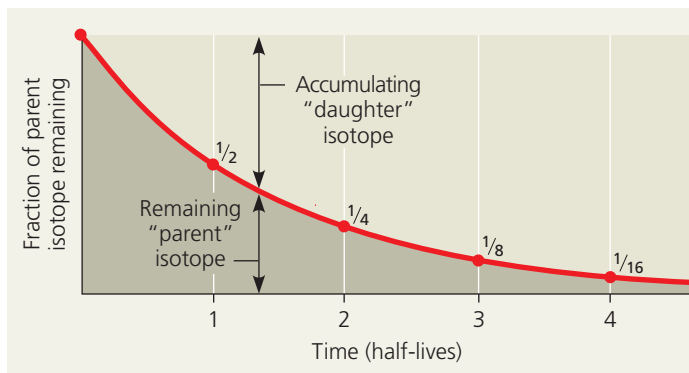
The Origin of New Groups of Organisms

Some fossils provide a detailed look at the origin of new groups of organisms. Such fossils are central to our understanding of evolution; they illustrate how new features arise and how long it takes for such changes to occur. We’ll examine one such case here: the origin of mammals.

Along with amphibians and reptiles, mammals belong to the group of animals called *tetrapods* (from the Greek *tetra*, four, and *pod*, foot), named for having four limbs. Mammals have a number of unique anatomical features that fossilize readily, allowing scientists to trace their origin. For example, the lower jaw is composed of one bone (the dentary) in mammals but several bones in other tetrapods. In addition, the lower and upper jaws in mammals hinge between a different set of bones than in other tetrapods. Mammals also have a unique set of three bones that transmit sound in the middle ear, the hammer, anvil, and stirrup, whereas other tetrapods have only one such bone, the stirrup (see Concept 34.6). Finally, the teeth of mammals are differentiated into incisors (for tearing), canines (for piercing), and the multi-pointed premolars and molars (for crushing and grinding). In contrast, the teeth of other tetrapods usually consist of a row of undifferentiated, single-pointed teeth.

As detailed in Figure 25.7, the fossil record shows that the unique features of mammalian jaws and teeth evolved gradually over time, in a series of steps. As you study Figure 25.7, bear in mind that it includes just a few examples of the fossil skulls that document the origin of mammals. If all the known fossils in the sequence were arranged by shape and placed side by side, their features would blend smoothly from one group to the next. Some of these fossils would reflect how the features of a group that dominates life today, the mammals, gradually arose in a previously existing group, the cynodonts. Others would reveal side branches on the tree of life—groups of organisms that thrived for millions of years but ultimately left no descendants that survive today.

▼ **Figure 25.6 Radiometric dating.** In this diagram, each unit of time represents one half-life of a radioactive isotope.



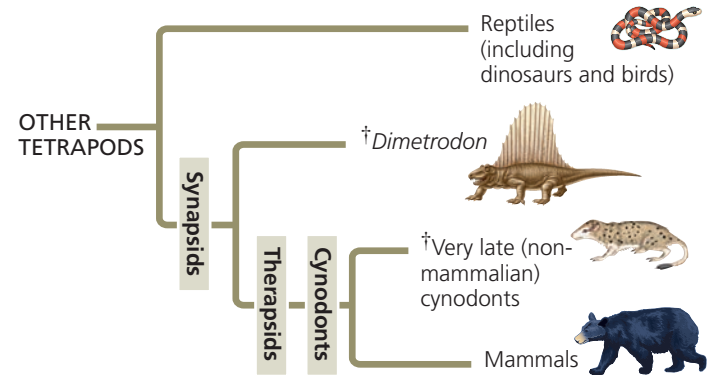
DRAW IT ► Relabel the x-axis of this graph in years to illustrate the radioactive decay of uranium-238 (half-life = 4.5 billion years).

▼ Figure 25.7 Exploring The Origin of Mammals

Over the course of 120 million years, mammals originated gradually from a group of tetrapods called synapsids. Shown here are a few of the many fossil organisms whose morphological features represent intermediate steps between living mammals and their early synapsid ancestors. The evolutionary context of the origin of mammals is shown in the tree diagram at right (the dagger symbol † indicates extinct lineages).

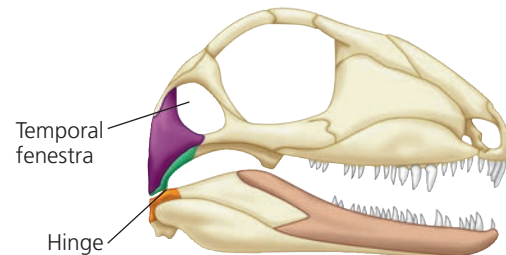
Key to skull bones

Articular	Dentary
Quadrate	Squamosal



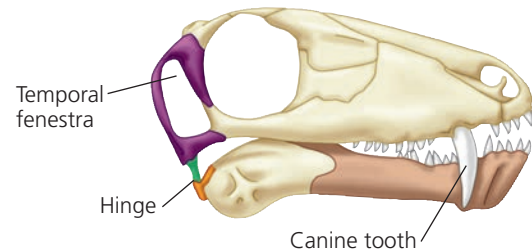
Synapsid (300 mya)

Early synapsids had multiple bones in the lower jaw and single-pointed teeth. The jaw hinge was formed by the articular and quadrate bones. Early synapsids also had an opening called the *temporal fenestra* behind the eye socket. Powerful cheek muscles for closing the jaws probably passed through the temporal fenestra. Over time, this opening enlarged and moved in front of the hinge between the lower and upper jaws, thereby increasing the power and precision with which the jaws could be closed (much as moving a doorknob away from the hinge makes a door easier to close).



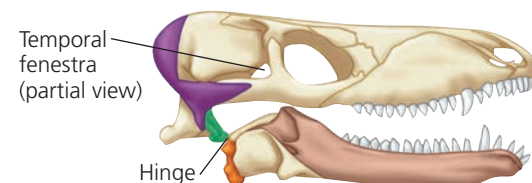
Therapsid (280 mya)

Later, a group of synapsids called therapsids appeared. Therapsids had large dentary bones, long faces, and the first examples of specialized teeth, large canines. These trends continued in a group of therapsids called cynodonts.



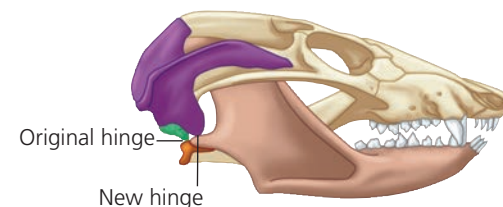
Early cynodont (260 mya)

In early cynodont therapsids, the dentary was the largest bone in the lower jaw, the temporal fenestra was large and positioned forward of the jaw hinge, and teeth with several cusps first appeared (not visible in the diagram). As in earlier synapsids, the jaw had an articular-quadrate hinge.



Later cynodont (220 mya)

Later cynodonts had teeth with complex cusp patterns, and their lower and upper jaws hinged in two locations: They retained the original articular-quadrate hinge and formed a new, second hinge between the dentary and squamosal bones. (The temporal fenestra is not visible in this or the below cynodont skull at the angles shown.)



Very late cynodont (195 mya)

In some very late (nonmammalian) cynodonts and early mammals, the original articular-quadrate hinge was lost, leaving the dentary-squamosal hinge as the only hinge between the lower and upper jaws, as in living mammals. The articular and quadrate bones migrated into the ear region (not shown), where they functioned in transmitting sound. In the mammal lineage, these two bones later evolved into the familiar hammer (malleus) and anvil (incus) bones of the ear.



CONCEPT CHECK 25.2

1. Describe an example from the fossil record that shows how life has changed over time.
2. **WHAT IF? >** Your measurements indicate that a fossilized skull you unearthed has a carbon-14/carbon-12 ratio about $\frac{1}{16}$ that of the skulls of present-day animals. What is the approximate age of the fossilized skull?

For suggested answers, see Appendix A.

CONCEPT 25.3

Key events in life's history include the origins of unicellular and multicellular organisms and the colonization of land

The study of fossils has helped geologists establish a **geologic record**: a standard time scale that divides Earth's history into four eons and further subdivisions (**Table 25.1**). The

first three eons—the Hadean, Archaean, and Proterozoic—together lasted about 4 billion years. The Phanerozoic eon, roughly the last half billion years, encompasses most of the time that animals have existed on Earth. It is divided into three eras: the Paleozoic, Mesozoic, and Cenozoic. Each era represents a distinct age in the history of Earth and its life. For example, the Mesozoic era is sometimes called the “age of reptiles” because of its abundance of reptilian fossils, including those of dinosaurs. The boundaries between the eras correspond to major extinction events, when many forms of life disappeared and were replaced by forms that evolved from the survivors.

As we've seen, the fossil record provides a sweeping overview of the history of life over geologic time. Here we will focus on a few major events in that history, returning to study the details in Unit Five. **Figure 25.8** will help you visualize how long ago these key events occurred against the vast backdrop of geologic time.

▼ Figure 25.8 Visualizing the Scale of Geologic Time

Geologic time is so vast that it can be difficult to visualize when key events in the history of life on Earth occurred. This figure introduces two common representations that help place the timing of those events in context: a countdown timer and a horizontal time line.

Using the analogy of a timer that begins with the origin of Earth and counts down for one hour, we can relate the relative timing and duration of events that occurred billions of years ago to a familiar time scale. On a one-hour time scale, animals originated about 9 minutes ago, while humans appeared less than 0.2 seconds ago.

This diagram “uncoils” the timer to represent life's history on a horizontal time line. Time runs from left to right, from 4.6 billion years ago to the present. The color-coding will help you relate these diagrams to each other and to Table 25.1.

Origin of solar system and Earth

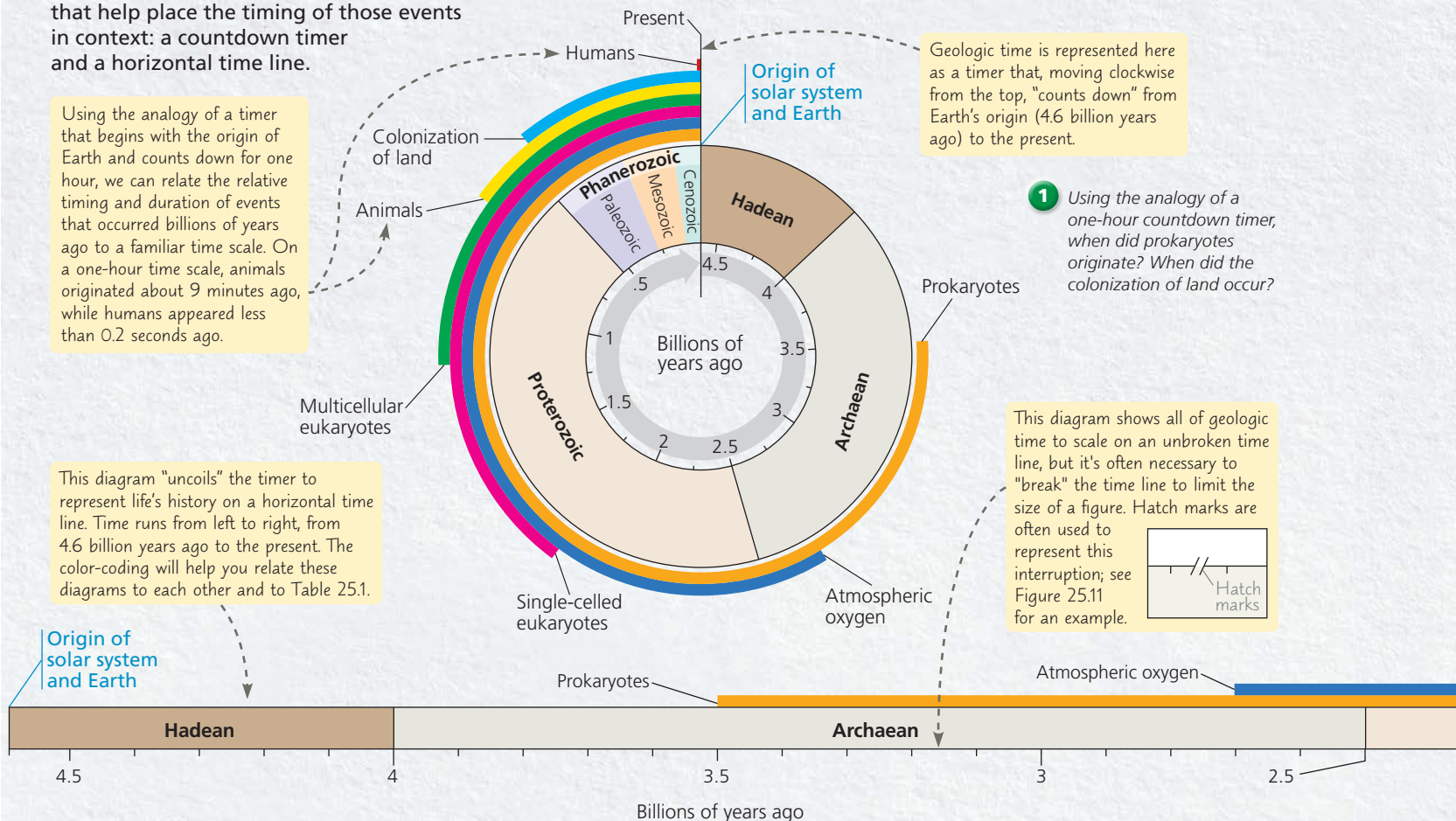
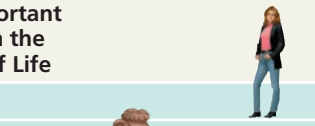
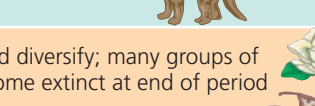
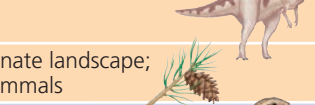
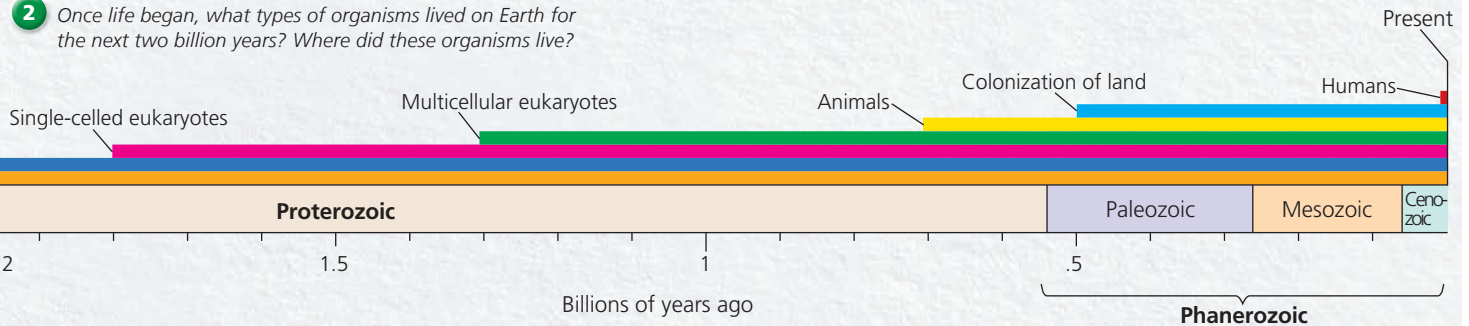


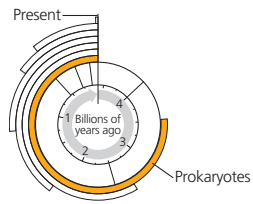
Table 25.1 The Geologic Record

Eons (duration not to scale)	Era	Period	Epoch	Age (Millions of Years Ago)	Some Important Events in the History of Life	
Phan- erozoic	Cenozoic	Quaternary	Holocene	0.01	Historical time	
			Pleistocene	2.6	Ice ages; origin of genus <i>Homo</i>	
		Neogene	Pliocene	5.3	Appearance of bipedal human ancestors	
			Miocene	23	Continued radiation of mammals and angiosperms; earliest direct human ancestors	
		Paleogene	Oligocene	34	Origins of many primate groups	
			Eocene	56	Angiosperm dominance increases; continued radiation of most present-day mammalian orders	
			Paleocene	66	Major radiation of mammals, birds, and pollinating insects	
	Mesozoic	Cretaceous	145	Flowering plants (angiosperms) appear and diversify; many groups of organisms, including most dinosaurs, become extinct at end of period		
		Jurassic	201	Gymnosperms continue as dominant plants; dinosaurs abundant and diverse		
		Triassic	252	Cone-bearing plants (gymnosperms) dominate landscape; dinosaurs evolve and radiate; origin of mammals		
Paleozoic	Permian		299	Radiation of reptiles; origin of most present-day groups of insects; extinction of many marine and terrestrial organisms at end of period		
		Carboniferous	359	Extensive forests of vascular plants form; first seed plants appear; origin of reptiles; amphibians dominant		
	Devonian		419	Diversification of bony fishes; first tetrapods and insects appear		
		Silurian	444	Diversification of early vascular plants		
	Ordovician		485	Marine algae abundant; colonization of land by diverse fungi, plants, and animals		
		Cambrian	541	Sudden increase in diversity of many animal phyla (Cambrian explosion)		
	Proter- ozoic	Neo- proterozoic	Ediacaran		541	Diverse algae and soft-bodied invertebrate animals appear
				635	Oldest fossils of eukaryotic cells appear	
				1,000	Oldest known rocks on Earth's surface	
Archean				1,800	Concentration of atmospheric oxygen begins to increase	
				2,500	Oldest fossils of cells (prokaryotes) appear	
				2,700	Oldest known rocks on Earth's surface	
Hadean				Approx. 4,600	Origin of Earth	

2 Once life began, what types of organisms lived on Earth for the next two billion years? Where did these organisms live?



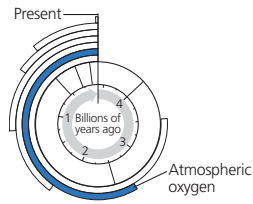
The First Single-Celled Organisms



The earliest direct evidence of life, dating from 3.5 billion years ago, comes from fossilized stromatolites (see Figure 25.5). **Stromatolites** are layered rocks that form when certain prokaryotes bind thin films of

sediment together. Stromatolites and other early prokaryotes were Earth's sole inhabitants for about 1.5 billion years. As we will see, these prokaryotes transformed life on our planet.

Photosynthesis and the Oxygen Revolution

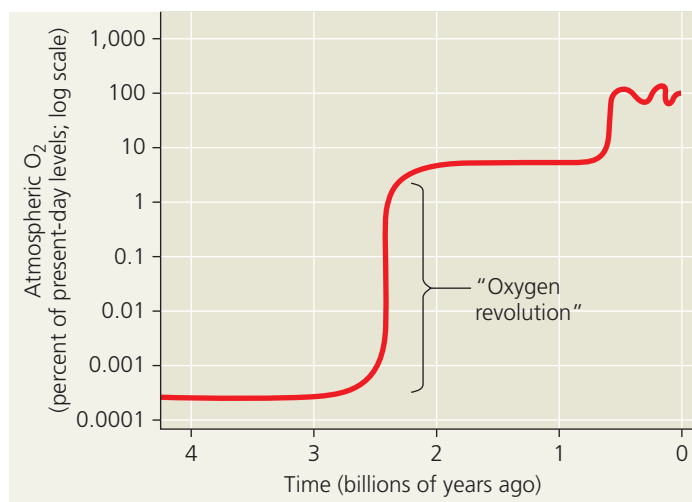


Most atmospheric oxygen gas (O_2) is of biological origin, produced during the water-splitting step of photosynthesis. When oxygenic photosynthesis first evolved—in photosynthetic prokaryotes—the free O_2 it produced

probably dissolved in the surrounding water until it reached a high enough concentration to react with elements dissolved in water, including iron. This would have caused the iron to precipitate as iron oxide, which accumulated as sediments. These sediments were compressed into banded iron formations, red layers of rock containing iron oxide that are a source of iron ore today. Once all of the dissolved iron had precipitated, additional O_2 dissolved in the water until the seas and lakes became saturated with O_2 . After this occurred, the O_2 finally began to “gas out” of the water and enter the atmosphere. This change left its mark in the rusting of iron-rich terrestrial rocks, a process that began about 2.7 billion years ago. This chronology implies that bacteria similar to today's cyanobacteria (oxygen-releasing, photosynthetic bacteria) originated before 2.7 billion years ago.

As shown in **Figure 25.9**, the amount of atmospheric O_2 increased gradually from about 2.7 to 2.4 billion years ago,

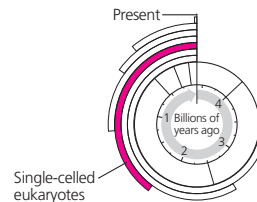
✓ Figure 25.9 The rise of atmospheric oxygen. Chemical analyses of ancient rocks have enabled this reconstruction of atmospheric oxygen levels during Earth's history.



but then shot up relatively rapidly to between 1% and 10% of its present level. This “oxygen revolution” had an enormous impact on life. In some of its chemical forms, oxygen attacks chemical bonds and can inhibit enzymes and damage cells. As a result, the rising concentration of atmospheric O_2 probably doomed many prokaryotic groups. Some species survived in habitats that remained anaerobic, where we find their descendants living today (see Concept 27.4). Among other survivors, diverse adaptations to the changing atmosphere evolved, including cellular respiration, which uses O_2 in the process of harvesting the energy stored in organic molecules.

The rise in atmospheric O_2 levels left a huge imprint on the history of life. A few hundred million years later, another fundamental change occurred: the origin of the eukaryotic cell.

The First Eukaryotes



The oldest widely accepted fossils of eukaryotic organisms are 1.8 billion years old. Recall that eukaryotic cells have more complex organization than prokaryotic cells: Eukaryotic cells have a nuclear envelope, mitochondria, endoplasmic reticulum, and other internal structures that prokaryotes lack. Also, unlike prokaryotic cells, eukaryotic cells have a well-developed cytoskeleton, a feature that enables eukaryotic cells to change their shape and thereby surround and engulf other cells.

How did the eukaryotes evolve from their prokaryotic ancestors? Current evidence indicates that the eukaryotes originated by **endosymbiosis** when a prokaryotic cell engulfed a small cell that would evolve into an organelle found in all eukaryotes, the mitochondrion. The small, engulfed cell is an example of an *endosymbiont*, a cell that lives within another cell, called the *host cell*. The prokaryotic ancestor of the mitochondrion probably entered the host cell as undigested prey or an internal parasite. Though such a process may seem unlikely, scientists have directly observed cases in which endosymbionts that began as prey or parasites developed a mutually beneficial relationship with the host in as little as five years.

By whatever means the relationship began, we can hypothesize how the symbiosis could have become beneficial. For example, in a world that was becoming increasingly aerobic, a host that was itself an anaerobe would have benefited from endosymbionts that could make use of the oxygen. Over time, the host and endosymbionts would have become a single organism, its parts inseparable. Although all eukaryotes have mitochondria or remnants of these organelles, they do not all have plastids (a general term for chloroplasts and related organelles). Thus, the **serial endosymbiosis** hypothesis supposes that mitochondria evolved before plastids through a sequence of endosymbiotic events. As shown in **Figure 25.10**, both

▼ **Figure 25.10 A hypothesis for the origin of mitochondria and plastids through serial endosymbiosis.** The proposed host was an archaean or a close relative of the archaeans. The proposed ancestors of mitochondria were aerobic, heterotrophic bacteria, while the proposed ancestors of plastids were photosynthetic bacteria. In this figure, the arrows represent change over evolutionary time.

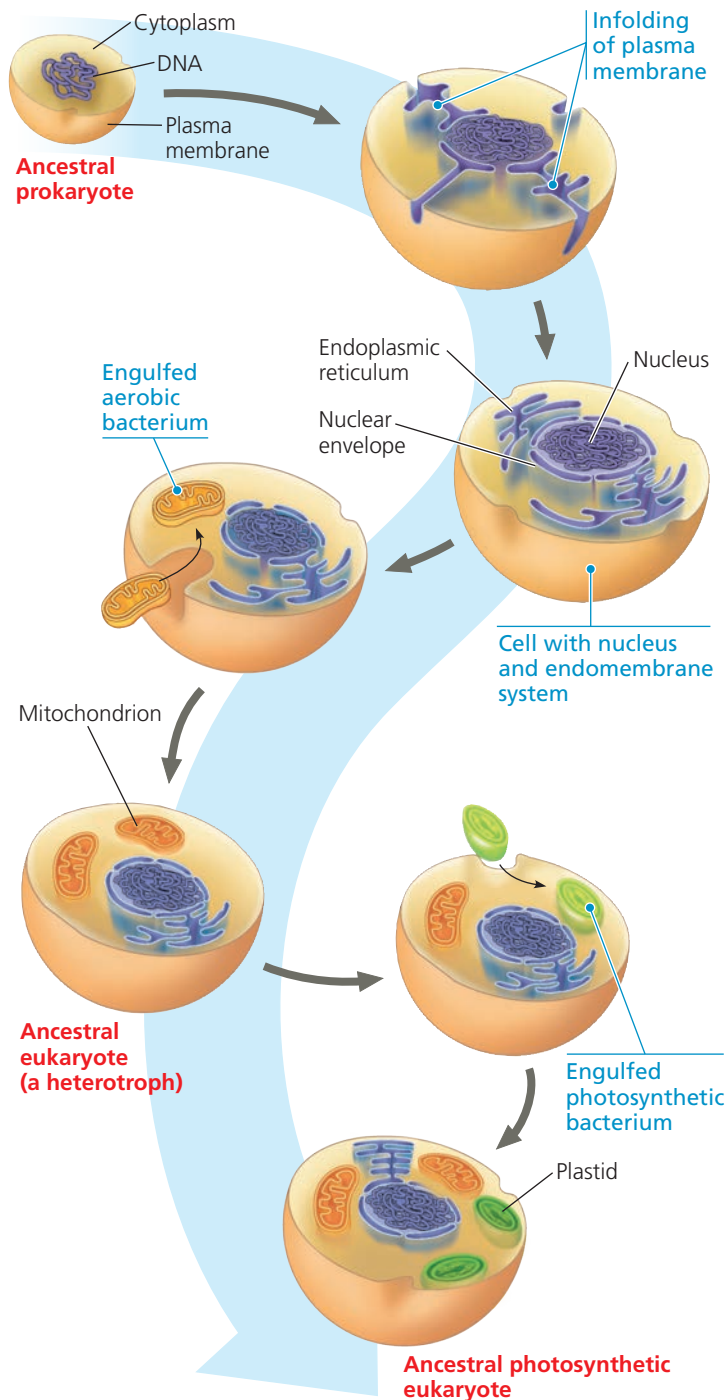


Figure Walkthrough

mitochondria and plastids are thought to have descended from bacterial cells. The original host—the cell that engulfed the bacterium whose descendants gave rise to the mitochondrion—is thought to have been an archaean or a close relative of the archaeans.

A great deal of evidence supports the endosymbiotic origin of mitochondria and plastids:

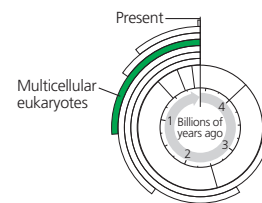
- The inner membranes of both organelles have enzymes and transport systems that are homologous to those found in the plasma membranes of living bacteria.
- Mitochondria and plastids replicate by a splitting process that is similar to that of certain bacteria. In addition, each of these organelles contains circular DNA molecules that, like the chromosomes of bacteria, are not associated with histones or large amounts of other proteins.
- As might be expected of organelles descended from free-living organisms, mitochondria and plastids also have the cellular machinery (including ribosomes) needed to transcribe and translate their DNA into proteins.
- Finally, in terms of size, RNA sequences, and sensitivity to certain antibiotics, the ribosomes of mitochondria and plastids are more similar to bacterial ribosomes than they are to the cytoplasmic ribosomes of eukaryotic cells.

In Chapter 28, we'll return to the origin of eukaryotes, focusing on what genomic data have revealed about the prokaryotic lineages that gave rise to the host and endosymbiont cells.

The Origin of Multicellularity

An orchestra can play a greater variety of musical compositions than a violin soloist can; the increased complexity of the orchestra makes more variations possible. Likewise, the origin of structurally complex eukaryotic cells sparked the evolution of greater morphological diversity than was possible for the simpler prokaryotic cells. After the first eukaryotes appeared, a great range of unicellular forms evolved, giving rise to the diversity of single-celled eukaryotes that continue to flourish today. Another wave of diversification also occurred: Some single-celled eukaryotes gave rise to multicellular forms, whose descendants include a variety of algae, plants, fungi, and animals.

Early Multicellular Eukaryotes



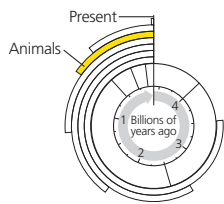
The oldest known fossils of multicellular eukaryotes that can be resolved taxonomically are of relatively small red algae that lived 1.2 billion years ago; even older fossils, dating to 1.8 billion years

ago, may also be of small, multicellular eukaryotes. Larger and more diverse multicellular eukaryotes do not appear in the fossil record until about 600 million years ago (see Figure 25.5). These fossils, referred to as the Ediacaran biota, were of soft-bodied organisms—some over 1 m long—that lived from 635 to 541 million years ago. The Ediacaran biota included both algae and animals, along with various organisms of unknown taxonomic affinity.

The rise of large eukaryotes in the Ediacaran period represents an enormous change in the history of life. Before

that time, Earth was a microbial world: Its only inhabitants were single-celled prokaryotes and eukaryotes, along with an assortment of microscopic, multicellular eukaryotes. As the diversification of the Ediacaran biota came to a close about 541 million years ago, the stage was set for another, even more spectacular burst of evolutionary change: the “Cambrian explosion.”

The Cambrian Explosion

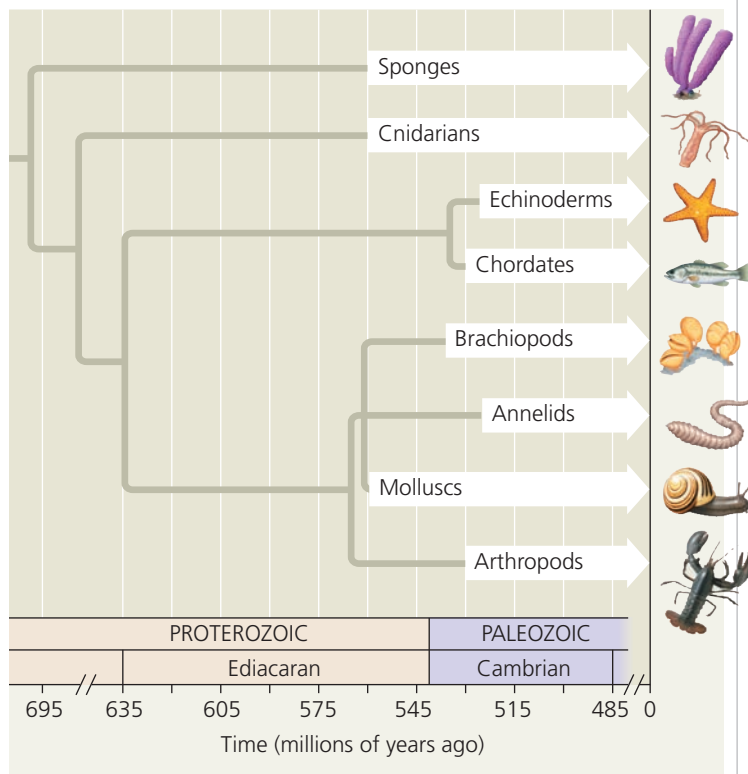


Many present-day animal phyla appear suddenly in fossils formed 535–525 million years ago, early in the Cambrian period. This phenomenon is referred to as the **Cambrian explosion**. Fossils of several animal

groups—sponges, cnidarians (sea anemones and their relatives), and molluscs (snails, clams, and their relatives)—appear in even older rocks dating from the late Proterozoic (**Figure 25.11**).

Prior to the Cambrian explosion, all large animals were soft-bodied. The fossils of large pre-Cambrian animals reveal little evidence of predation. Instead, these animals appear to have been grazers (feeding on algae), filter feeders, or scavengers, not hunters. The Cambrian explosion changed all of

▼ **Figure 25.11 Appearance of selected animal groups.** The white bars indicate earliest appearances of these animal groups in the fossil record.

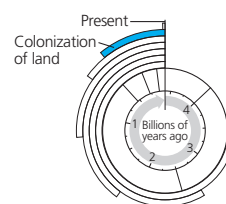


VISUAL SKILLS ► Circle the branch point that represents the most recent common ancestor of chordates and annelids. What is a minimum estimate of that ancestor’s age?

that. In a relatively short period of time (10 million years), predators over 1 m in length emerged that had claws and other features for capturing prey; simultaneously, new defensive adaptations, such as sharp spines and heavy body armor, appeared in their prey (see **Figure 25.5**).

Although the Cambrian explosion had an enormous impact on life on Earth, it appears that many animal phyla originated long before that time. Recent DNA analyses suggest that sponges had evolved by 700 million years ago; such analyses also indicate that the common ancestor of arthropods, chordates, and other animal phyla that radiated during the Cambrian explosion lived 670 million years ago. Researchers have unearthed 710-million-year-old sediments containing steroids indicative of a particular group of sponges—a finding that supports the molecular data. In contrast, the oldest fossil assigned to an extant animal phylum is that of the mollusc *Kimberella*, which lived 560 million years ago. Overall, molecular and fossil data indicate that the Cambrian explosion had a “long fuse”—at least 25 million years long based on the age of *Kimberella* fossils, and over 100 million years long based on some DNA analyses.

The Colonization of Land



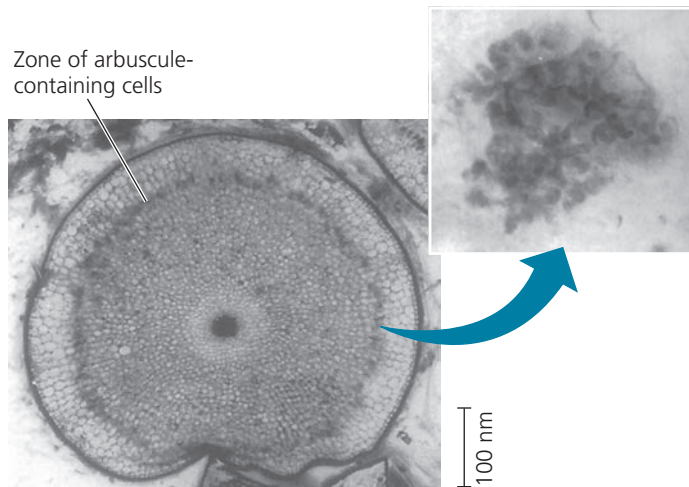
The colonization of land was another milestone in the history of life. There is fossil evidence that cyanobacteria and other photosynthetic prokaryotes coated damp terrestrial surfaces well over a billion years ago. However, larger forms of life, such as fungi,

plants, and animals, did not begin to colonize land until about 500 million years ago. This gradual evolutionary venture out of aquatic environments was associated with adaptations that made it possible to reproduce on land and that helped prevent dehydration. For example, many land plants today have a vascular system for transporting materials internally and a waterproof coating of wax on their leaves that slows the loss of water to the air. Early signs of these adaptations were present 420 million years ago, at which time small plants (about 10 cm high) existed that had a vascular system but lacked true roots or leaves. By 40 million years later, plants had diversified greatly and included reeds and treelike plants with true roots and leaves.

Plants appear to have colonized land in the company of fungi. Even today, the roots of most plants are associated with fungi that aid in the absorption of water and minerals from the soil (see **Concept 31.1**). These root fungi (or *mycorrhizae*), in turn, obtain their organic nutrients from the plants. Such mutually beneficial associations of plants and fungi are evident in some of the oldest fossilized plants, dating this relationship back to the early spread of life onto land (**Figure 25.12**).

Although many animal groups are now represented in terrestrial environments, the most widespread and diverse

▼ **Figure 25.12 An ancient symbiosis.** This 405-million-year-old fossil stem (cross section) documents mycorrhizae in the early land plant *Aglaophyton major*. The inset shows an enlarged view of a cell containing a branched fungal structure called an arbuscule; the fossil arbuscule resembles those seen in plant cells today.



land animals are arthropods (particularly insects and spiders) and tetrapods. Arthropods were among the first animals to colonize land, roughly 450 million years ago. The earliest tetrapods found in the fossil record lived about 365 million years ago and appear to have evolved from a group of lobe-finned fishes (see Concept 34.3). Tetrapods include humans, although we are late arrivals on the scene. The human lineage diverged from other primates around 6–7 million years ago, and our species originated only about 195,000 years ago. If the clock of Earth's history were rescaled to represent an hour, humans appeared less than 0.2 second ago.



Interview with Geerat Vermeij: What fossils reveal about the history of life

CONCEPT CHECK 25.3

1. The first appearance of free oxygen in the atmosphere likely triggered a massive wave of extinctions among the prokaryotes of the time. Why?
2. What evidence supports the hypothesis that mitochondria preceded plastids in the evolution of eukaryotic cells?
3. **WHAT IF? >** What would a fossil record of life today look like?

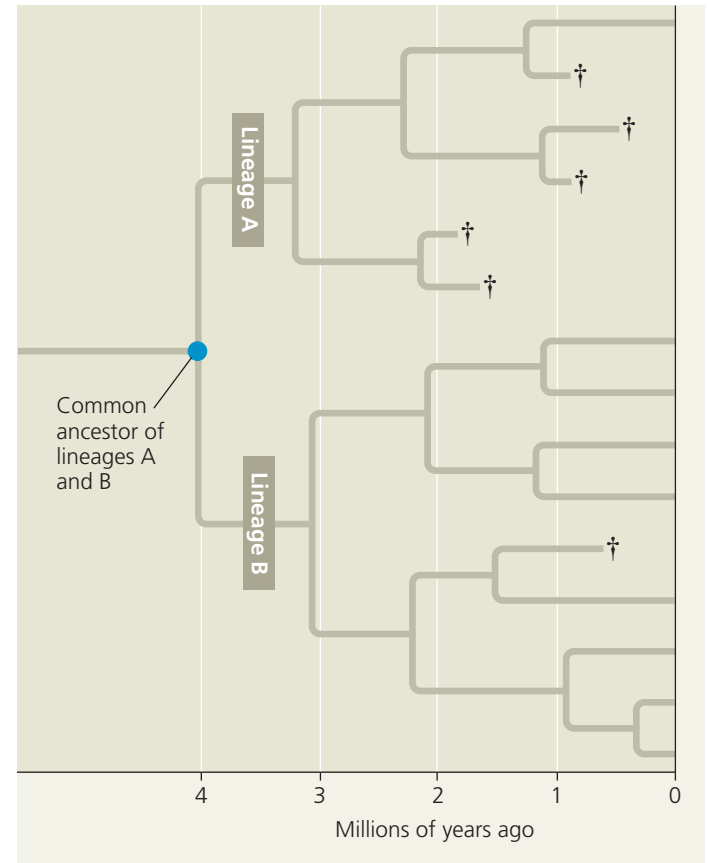
For suggested answers, see Appendix A.

CONCEPT 25.4

The rise and fall of groups of organisms reflect differences in speciation and extinction rates

From its beginnings, life on Earth has been marked by the rise and fall of groups of organisms. Anaerobic prokaryotes originated, flourished, and then declined as the oxygen content of the atmosphere rose. Billions of years later, the first tetrapods

▼ **Figure 25.13 How speciation and extinction affect diversity.** The species diversity of an evolutionary lineage will increase when more new member species originate than are lost to extinction. In this hypothetical example, by 2 million years ago both lineage A and lineage B have given rise to four species, and no species have become extinct. Over the next 2 million years, however, lineage A experiences higher extinction rates than does lineage B (extinct species are denoted by a dagger symbol, †). As a result, after 4 million years (that is, by time 0), lineage A contains only one species, while lineage B contains eight species.



emerged from the sea, giving rise to several major new groups of organisms. One of these, the amphibians, went on to dominate life on land for 100 million years, until other tetrapods (including dinosaurs and, later, mammals) replaced them as the dominant terrestrial vertebrates.

The rise and fall of these and other major groups of organisms have shaped the history of life. Narrowing our focus, we can also see that the rise or fall of any particular group is related to the speciation and extinction rates of its member species (**Figure 25.13**). Just as a population increases in size when there are more births than deaths, the rise of a group of organisms occurs when more new species are produced than are lost to extinction. The reverse occurs when a group is in decline. In the **Scientific Skills Exercise**, you will interpret data from the fossil record about changes in a group of snail species in the early Paleogene period. Such changes in the fates of groups of organisms have been influenced by large-scale processes such as plate tectonics, mass extinctions, and adaptive radiations.

SCIENTIFIC SKILLS EXERCISE

Estimating Quantitative Data from a Graph and Developing Hypotheses

Do Ecological Factors Affect Evolutionary Rates?

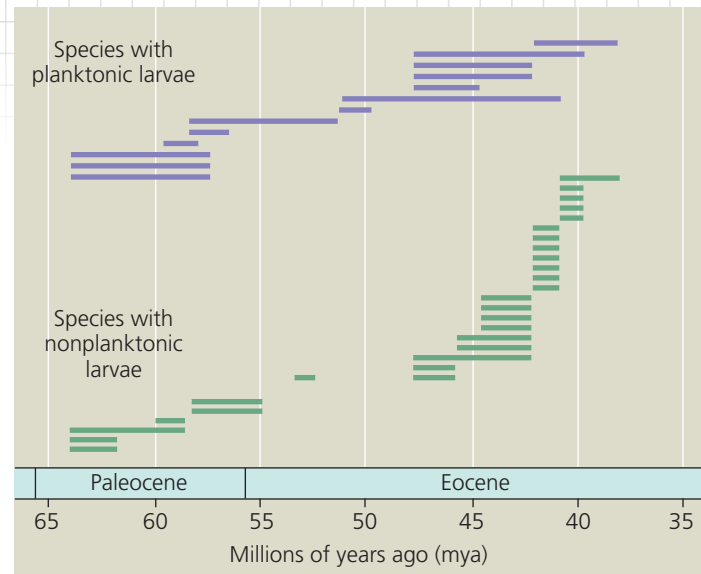
Researchers studied the fossil record to investigate whether differing modes of larval dispersal might explain species longevity within one taxon of marine snails, the family Volutidae. Some of the snail species had nonplanktonic larvae: They developed directly into adults without a swimming stage. Other species had planktonic larvae: They had a swimming stage and could disperse very long distances. The adults of these planktonic species tended to have broad geographic distributions, whereas nonplanktonic species tended to be more isolated.



How the Research Was Done The researchers studied the stratigraphic distribution of volutes in outcrops of sedimentary rocks located along North America's Gulf coast. These rocks, which formed from 66 to 37 million years ago, early in the Paleogene period, are an excellent source of well-preserved snail fossils. The researchers were able to classify each fossil species of volute snail as having planktonic or nonplanktonic larvae based on features of the earliest formed whorls of the snail's shell. Each bar in the graph shows how long one species of snail persisted in the fossil record.

INTERPRET THE DATA

1. You can estimate quantitative data (fairly precisely) from a graph. The first step is to obtain a conversion factor by measuring along an axis that has a scale. In this case, 25 million years (my; from 60 to 35 million years ago [mya] on the x-axis) is represented by a distance of 7.0 cm. This yields a conversion factor (a ratio) of $25 \text{ my}/7.0 \text{ cm} = 3.6 \text{ my/cm}$. To estimate the time period represented by a horizontal bar on this graph, measure the length of that bar in centimeters and multiply that measurement by the conversion factor, 3.6 my/cm .



For example, a bar that measures 1.1 cm on the graph represents a persistence time of $1.1 \text{ cm} \times 3.6 \text{ my/cm} = 4 \text{ million years}$.

2. Calculate the mean (average) persistence times for species with planktonic larvae and species with nonplanktonic larvae.
3. Count the number of new species that form in each group beginning at 60 mya (the first three species in each group were present around 64 mya, the first time period sampled, so we don't know when those species first appear in the fossil record).
4. Propose a hypothesis to explain the differences in longevity of snail species with planktonic and nonplanktonic larvae.

Data from T. A. Hansen, Larval dispersal and species longevity in Lower Tertiary gastropods, *Science* 199:885–887 (1978). Reprinted with permission from AAAS.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Plate Tectonics

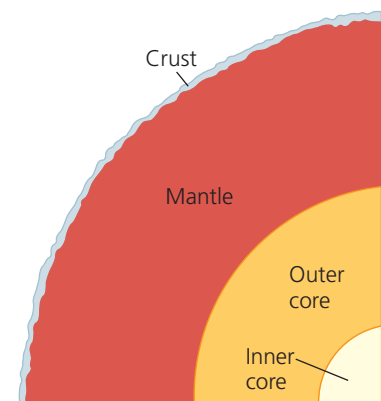
If photographs of Earth were taken from space every 10,000 years and spliced together to make a movie, it would show something many of us find hard to imagine: The seemingly “rock solid” continents we live on move over time. Over the past billion years, there have been three occasions (1 billion, 600 million, and 250 million years ago) when most of the landmasses of Earth came together to form a supercontinent, then later broke apart. Each time, this breakup yielded a different configuration of continents. Based on the directions in which the continents are moving today, some geologists have estimated that a new supercontinent will form roughly 250 million years from now.

According to the theory of **plate tectonics**, the continents are part of great plates of Earth's crust that essentially float on the hot, underlying portion of the mantle (**Figure 25.14**). Movements in the mantle cause the plates to move over time in a process called *continental drift*. Geologists can measure the rate at which the plates are moving now, usually only a few

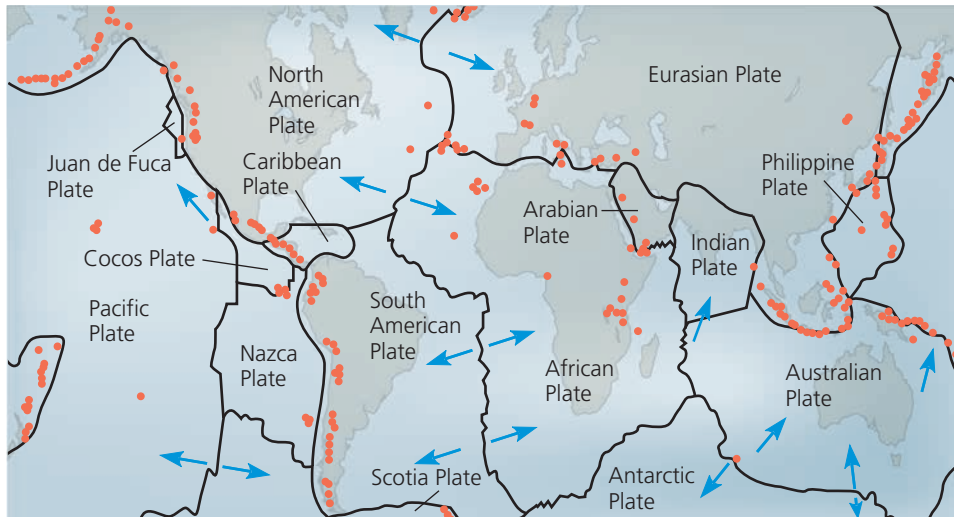
centimeters per year. They can also infer the past locations of the continents using the magnetic signal recorded in rocks at the time of their formation. This method works because as a continent shifts its position over time, the direction of magnetic north recorded in its newly formed rocks also changes.

Earth's major tectonic plates are shown in **Figure 25.15**. Many important geologic processes, including the formation of mountains and islands, occur at plate boundaries. In some cases, two plates are moving away from each other, as are the North American and Eurasian

Figure 25.14 Cutaway view of Earth. The thickness of the crust is exaggerated here.



▼ **Figure 25.15 Earth's major tectonic plates.** The arrows indicate direction of movement. The reddish orange dots represent zones of violent tectonic activity.



plates, which are currently drifting apart at a rate of about 2 cm per year. In other cases, two plates are sliding past each other, forming regions where earthquakes are common. California's infamous San Andreas Fault is part of a border where two plates slide past each other. In still other cases, two plates collide, producing violent upheavals and forming new mountains along the plate boundaries. One spectacular example of this occurred 45 million years ago, when the Indian plate crashed into the Eurasian plate, starting the formation of the Himalayan mountains.



Consequences of Continental Drift

Plate movements rearrange geography slowly, but their cumulative effects are dramatic. In addition to reshaping the physical features of our planet, continental drift also has a major impact on life on Earth.

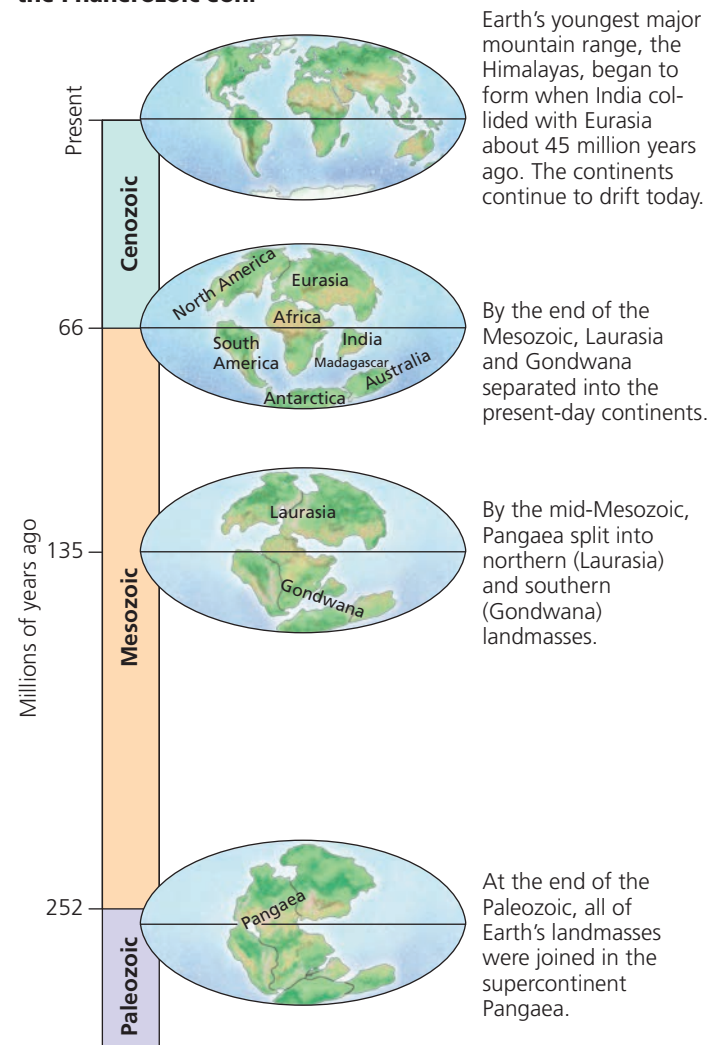
One reason for this is that continental drift alters the habitats in which organisms live. Consider the changes shown in **Figure 25.16**. About 250 million years ago, plate movements brought previously separated landmasses together into a supercontinent named **Pangaea**. Ocean basins became deeper, which drained shallow coastal seas. At that time, as now, most marine species inhabited shallow waters, and the formation of Pangaea destroyed much of that habitat. Pangaea's interior was cold and dry, probably an even more severe environment than that of central Asia today. Overall, the formation of Pangaea greatly altered the physical environment and climate, which drove some species to extinction and provided new opportunities for groups of organisms that survived the crisis.

Organisms are also affected by the climate change that results when a continent shifts its location. The southern tip of Labrador, Canada, for example, once was located in

the tropics but has moved 40° to the north over the last 200 million years. When faced with the changes in climate that such shifts in position entail, organisms adapt, move to a new location, or become extinct (this last outcome occurred for many organisms stranded on Antarctica, which separated from Australia 40 million years ago).

Continental drift also promotes allopatric speciation on a grand scale. When supercontinents break apart, regions that once were connected become isolated. As the continents drifted apart over the last 200 million years, each became a separate evolutionary arena, with lineages of plants and animals that diverged from those on other continents.

▼ **Figure 25.16 The history of continental drift during the Phanerozoic eon.**



VISUAL SKILLS ► Is the Australian plate's current direction of movement (see Figure 25.15) similar to the direction it traveled over the past 66 million years?

Finally, continental drift can help explain puzzles about the geographic distribution of extinct organisms, such as why fossils of the same species of Permian freshwater reptiles have been discovered in both Brazil and the West African nation of Ghana. These two parts of the world, now separated by 3,000 km of ocean, were joined together when these reptiles were living. Continental drift also explains much about the current distributions of organisms, such as why Australian fauna and flora contrast so sharply with those of the rest of the world. Marsupial mammals fill ecological roles in Australia analogous to those filled by eutherians (placental mammals) on other continents (see Figure 21.18). Fossil evidence suggests that marsupials originated in what is now Asia and reached Australia via South America and Antarctica while the continents were still joined. The subsequent breakup of the southern continents set Australia “afloat,” like a giant raft of marsupials. In Australia, marsupials diversified, and the few eutherians that lived there became extinct; on other continents, most marsupials became extinct, and the eutherians diversified.

Mass Extinctions

The fossil record shows that the overwhelming majority of species that ever lived are now extinct. A species may become extinct for many reasons. Its habitat may have been destroyed, or its environment may have changed in a manner unfavorable to the species. For example, if ocean temperatures fall by even a few degrees, species that are otherwise well adapted may perish. Even if physical factors in the environment remain stable, biological factors may change—the origin of one species can spell doom for another.

Although extinction occurs regularly, at certain times disruptive changes to the global environment have caused the rate of extinction to increase dramatically. The result is a **mass extinction**, in which large numbers of species become extinct worldwide.

The “Big Five” Mass Extinction Events

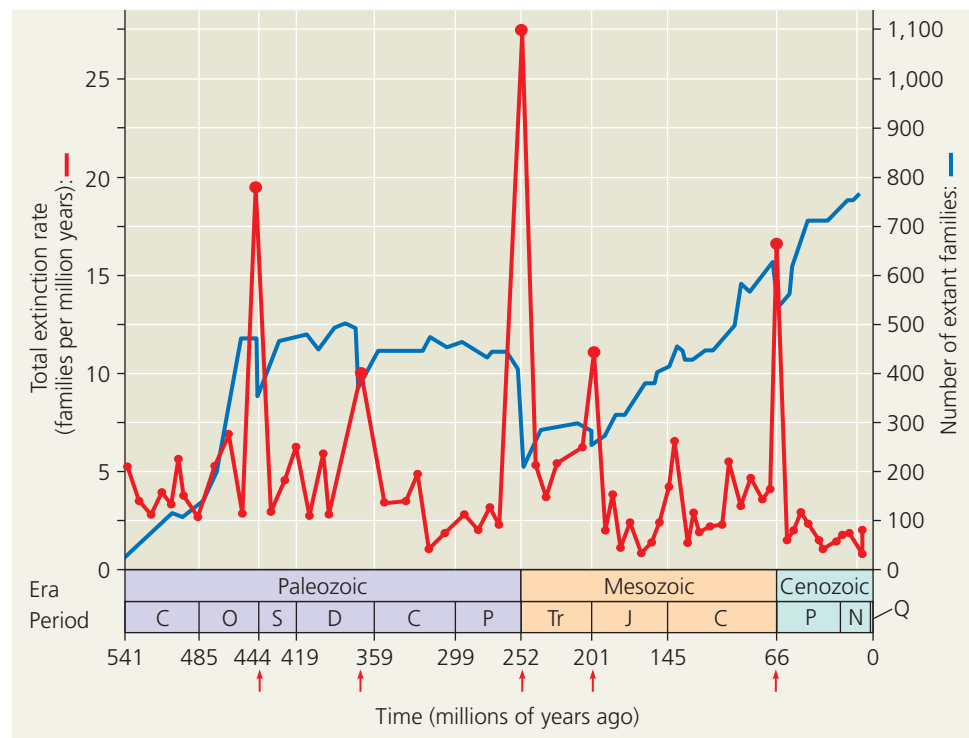
Five mass extinctions are documented in the fossil record over the past 500 million years (Figure 25.17). These events are particularly well documented for the decimation of hard-bodied animals that lived in shallow seas, the organisms for which the fossil record is most complete. In each mass extinction, 50% or more of marine species became extinct.

Two mass extinctions—the Permian and the Cretaceous—have received the most attention. The Permian

mass extinction, which defines the boundary between the Paleozoic and Mesozoic eras (252 million years ago), claimed about 96% of marine animal species and drastically altered life in the ocean. Terrestrial life was also affected. For example, 8 out of 27 known orders of insects were wiped out. This mass extinction occurred in less than 500,000 years, possibly in just a few thousand years—an instant in the context of geologic time.

The Permian mass extinction occurred during the most extreme episode of volcanism in the past 500 million years. Geologic data indicate that 1.6 million km² (roughly half the size of Western Europe) in Siberia was covered with lava hundreds of meters thick. The eruptions are thought to have produced enough carbon dioxide to warm the global climate by an estimated 6°C, harming many temperature-sensitive species. The rise in atmospheric CO₂ levels would also have led to ocean acidification, thereby reducing the availability of calcium carbonate, which is required by reef-building corals and many shell-building species (see Figure 3.12). The explosions would also have added nutrients such as phosphorus to marine ecosystems, stimulating the growth of microorganisms. Upon their deaths, these microorganisms would have provided food for bacterial decomposers. Bacteria use oxygen as they decompose the bodies of dead organisms, thus causing oxygen concentrations to drop. This would have harmed

▼ **Figure 25.17 Mass extinction and the diversity of life.** The five generally recognized mass extinction events, indicated by red arrows, represent peaks in the extinction rate of marine animal families (red line and left vertical axis). These mass extinctions interrupted the overall increase, over time, in the number of extant families of marine animals (blue line and right vertical axis).



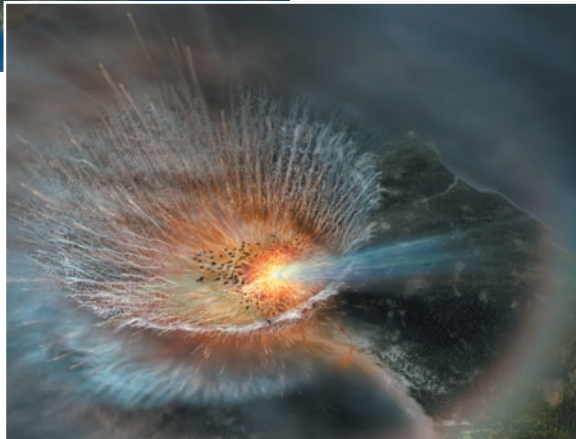
INTERPRET THE DATA ▶ As mentioned in the text, 96% of marine animal species became extinct in the Permian mass extinction. Explain why the blue curve shows only a 50% drop at that time.

oxygen-breathers and promoted the growth of anaerobic bacteria that emit a poisonous metabolic by-product, hydrogen sulfide (H_2S) gas. Overall, the volcanic eruptions may have triggered a series of catastrophic events that together resulted in the Permian mass extinction.

The Cretaceous mass extinction occurred 66 million years ago. This event extinguished more than half of all marine species and eliminated many families of terrestrial plants and animals, including all dinosaurs (except birds, which are members of the same group; see Figure 34.25). One clue to a possible cause of the Cretaceous mass extinction is a thin layer of clay enriched in iridium that dates to the time of the mass extinction. Iridium is an element that is very rare on Earth but common in many of the meteorites and other extraterrestrial objects that occasionally fall to Earth. As a result, researchers proposed that this clay is fallout from a huge cloud of debris that billowed into the atmosphere when an asteroid or large comet collided with Earth. This cloud would have blocked sunlight and severely disturbed the global climate for several months.

Is there evidence of such an asteroid or comet? Research has focused on the Chicxulub crater, a 66-million-year-old scar beneath sediments off the coast of Mexico (**Figure 25.18**).

▼ **Figure 25.18 A trauma for Cretaceous life.** Beneath the Caribbean Sea, the 66-million-year-old Chicxulub crater measures 180 km across. The horseshoe shape of the crater and the pattern of debris in sedimentary rocks indicate that an asteroid or comet struck at a low angle from the southeast. This drawing represents the impact and its immediate effect: a cloud of hot vapor and debris that could have killed many of the plants and animals in North America within hours.



The crater is the right size to have been caused by an object with a diameter of 10 km. Critical evaluation of this and other hypotheses for mass extinctions continues.



HHMI Video: The Day the Mesozoic Died

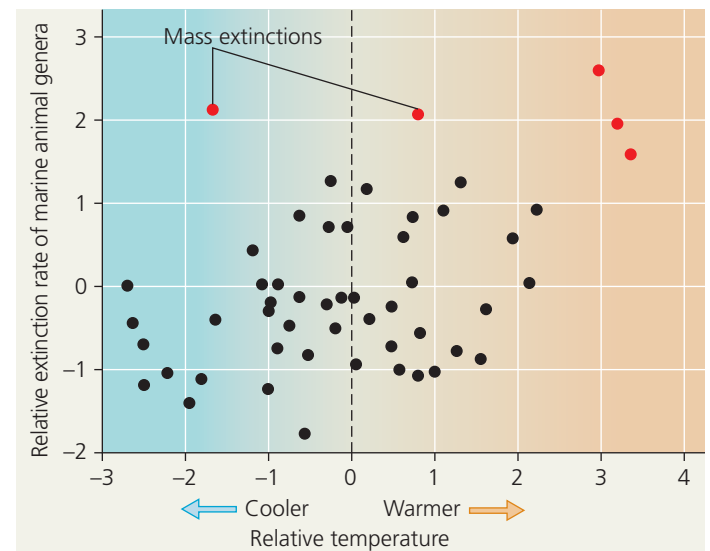


Is a Sixth Mass Extinction Under Way?

As you will read further in Concept 56.1, human actions, such as habitat destruction, are modifying the global environment to such an extent that many species are threatened with extinction. More than 1,000 species have become extinct in the last 400 years. Scientists estimate that this rate is 100 to 1,000 times the typical background rate seen in the fossil record. Is a sixth mass extinction now in progress?

This question is difficult to answer, in part because it is hard to document the total number of extinctions occurring today. Tropical rain forests, for example, harbor many undiscovered species. As a result, destroying tropical forest may drive species to extinction before we even learn of their existence. Such uncertainties make it hard to assess the full extent of the current extinction crisis. Even so, it is clear that losses to date have not reached those of the “big five” mass extinctions, in which large percentages of Earth’s species became extinct. This does not in any way discount the seriousness of today’s situation. Monitoring programs show that many species are declining at an alarming rate due to habitat loss, introduced species, overharvesting, and other factors. Recent studies on a variety of organisms, including lizards, pine trees, and polar bears, suggest that climate change may hasten some of these declines. The fossil record also highlights the potential importance of climate change: Over the last 500 million years, extinction rates have tended to increase when global temperatures were high (**Figure 25.19**).

▼ **Figure 25.19 Fossil extinctions and temperature.** Extinction rates increased when global temperatures were high. Temperatures were estimated using ratios of oxygen isotopes and converted to an index in which 0 is the overall average temperature.



Overall, the evidence suggests that unless dramatic actions are taken, a sixth, human-caused mass extinction is likely to occur within the next few centuries or millennia.

Consequences of Mass Extinctions

Mass extinctions have significant and long-term effects. By eliminating large numbers of species, a mass extinction can reduce a thriving and complex ecological community to a pale shadow of its former self. And once an evolutionary lineage disappears, it cannot reappear. The course of evolution is changed forever. Consider what would have happened if the early primates living 66 million years ago had died out in the Cretaceous mass extinction. Humans would not exist, and life on Earth would differ greatly from what it is today.

The fossil record shows that it typically takes 5–10 million years for the diversity of life to recover to previous levels after a mass extinction. In some cases, it has taken much longer than that: It took about 100 million years for the number of marine families to recover after the Permian mass extinction (see Figure 25.17). These data have sobering implications. If current trends continue and a sixth mass extinction occurs, it will take millions of years for life on Earth to recover.

Mass extinctions can also alter ecological communities by changing the types of organisms residing there. For example, after the Permian and Cretaceous mass extinctions, the percentage of marine organisms that were predators increased substantially (Figure 25.20). A rise in the number of predators can increase both the risks faced by prey and the competition among predators for food. In addition, mass extinctions can curtail lineages with novel and advantageous features. For example, in the late Triassic period, a group of gastropods (snails and their relatives) arose that could drill through the shells of bivalves (such as clams) and feed on the animals inside. Although shell

drilling provided access to a new and abundant source of food, this newly formed group was wiped out during the mass extinction at the end of the Triassic (about 200 million years ago). Another 120 million years passed before another group of gastropods (the oyster drills) exhibited the ability to drill through shells. As their predecessors might have done if they had not originated at an unfortunate time, oyster drills have since diversified into many new species. Finally, by eliminating so many species, mass extinctions can pave the way for adaptive radiations, in which new groups of organisms proliferate.

Adaptive Radiations

The fossil record shows that the diversity of life has increased over the past 250 million years (see blue line in Figure 25.17). This increase has been fueled by **adaptive radiations**, periods of evolutionary change in which groups of organisms form many new species whose adaptations allow them to fill different ecological roles, or niches, in their communities. Large-scale adaptive radiations occurred after each of the big five mass extinctions, when survivors became adapted to the many vacant ecological niches. Adaptive radiations have also occurred in groups of organisms that possessed major evolutionary innovations, such as seeds or armored body coverings, or that colonized regions in which they faced little competition from other species.

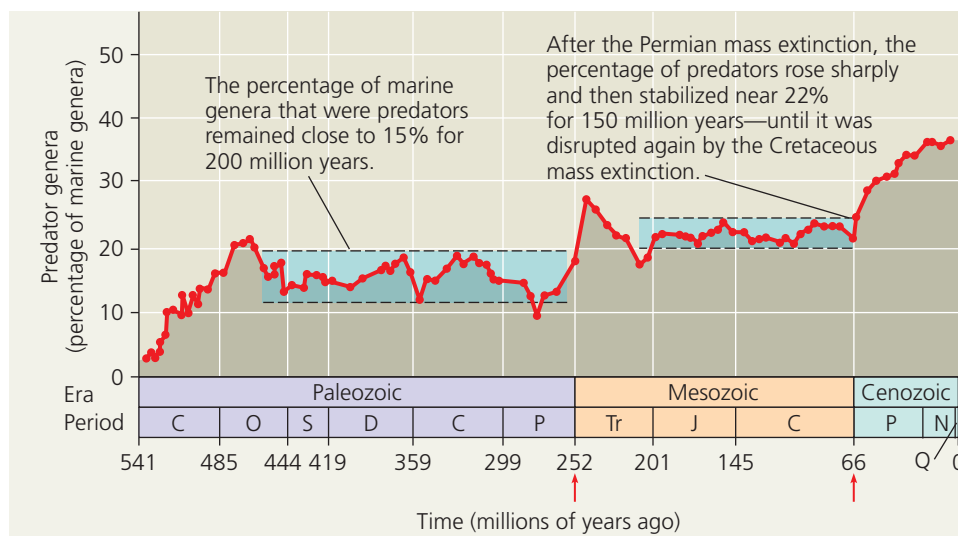
Worldwide Adaptive Radiations

Fossil evidence indicates that mammals underwent a dramatic adaptive radiation after the extinction of terrestrial dinosaurs 66 million years ago (Figure 25.21). Although mammals originated about 180 million years ago, the mammal fossils older than 66 million years are mostly small and not morphologically diverse. Many species appear to have been nocturnal

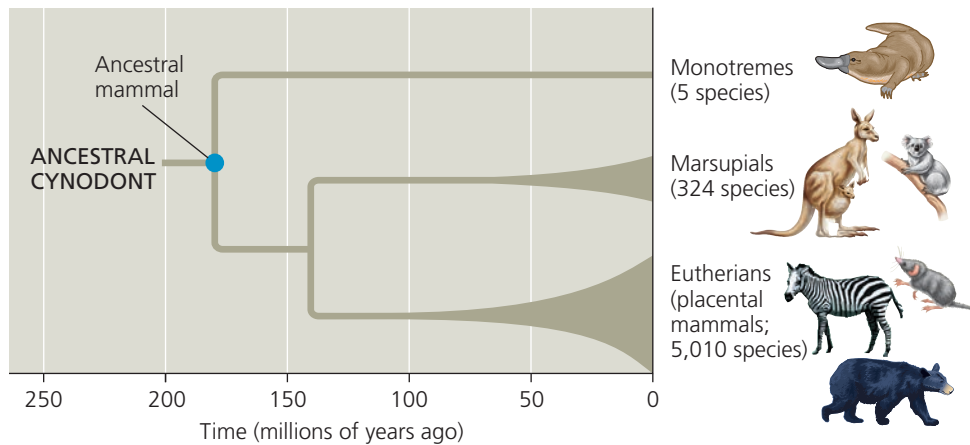
based on their large eye sockets, similar to those in living nocturnal mammals. A few early mammals were intermediate in size, such as *Repenomamus gigantiscus*, a 1-m-long predator that lived 130 million years ago—but none approached the size of many dinosaurs. Early mammals may have been restricted in size and diversity because they were eaten or outcompeted by the larger and more diverse dinosaurs. With the disappearance of the dinosaurs (except for birds), mammals expanded greatly in both diversity and size, filling the ecological roles once occupied by terrestrial dinosaurs.

The history of life has also been greatly altered by radiations in which groups of organisms increased in diversity as they came to play entirely new ecological roles in their communities. As we'll explore

▼ **Figure 25.20 Mass extinctions and ecology.** The Permian and Cretaceous mass extinctions (indicated by red arrows) altered the ecology of the oceans by increasing the percentage of marine genera that were predators.



▼ **Figure 25.21 Adaptive radiation of mammals.**



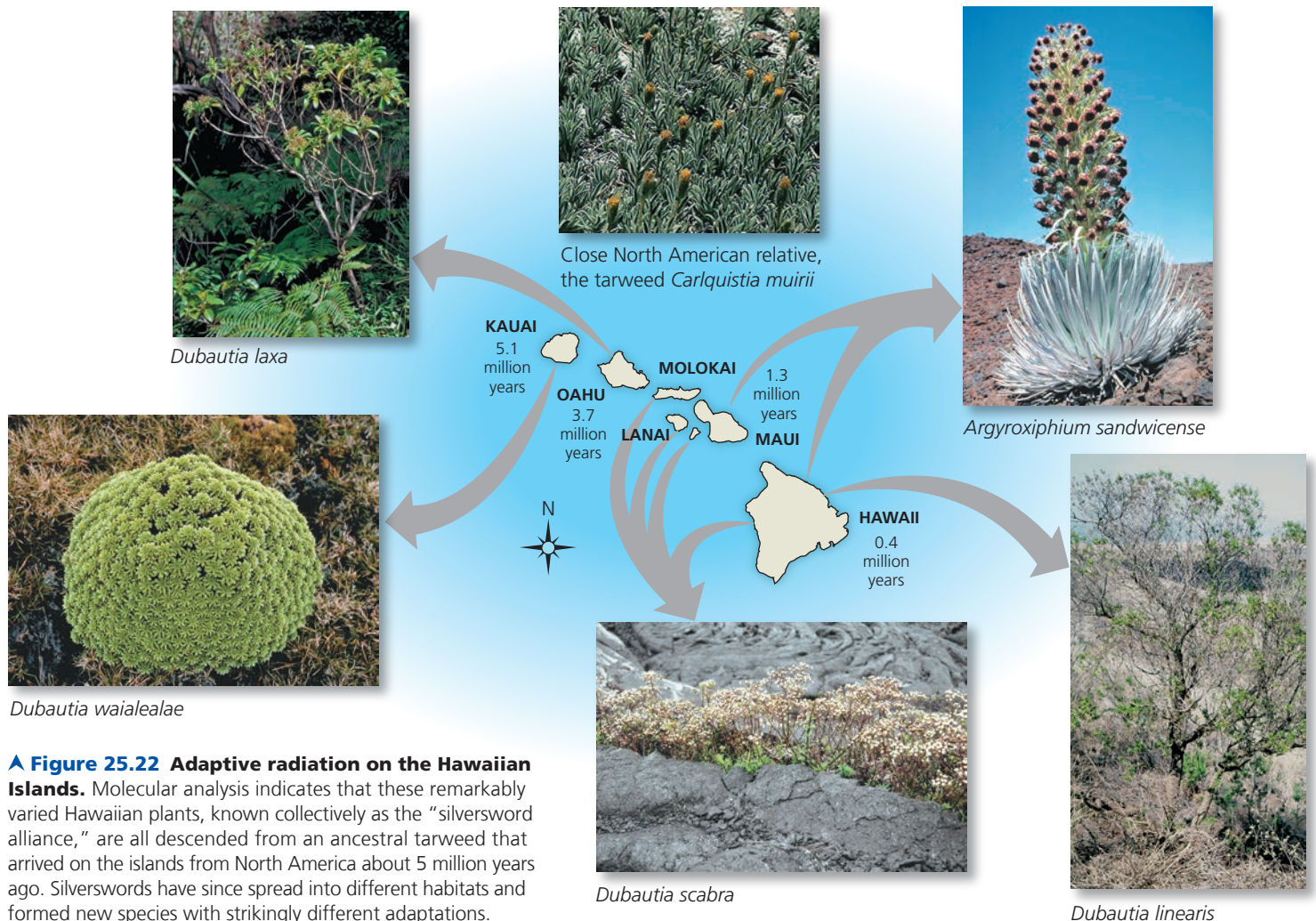
in later chapters, examples include the rise of photosynthetic prokaryotes, the evolution of large predators in the Cambrian explosion, and the radiations following the colonization of land by plants, insects, and tetrapods. Each of these last three radiations was associated with major evolutionary innovations that facilitated life on land. The radiation of land plants, for example, was associated with key adaptations, such as stems

that support plants against gravity and a waxy coat that protects leaves from water loss. Finally, organisms that arise in an adaptive radiation can serve as a new source of food for still other organisms. In fact, the diversification of land plants stimulated a series of adaptive radiations in insects that ate or pollinated plants, one reason that insects are the most diverse group of animals on Earth today.

Regional Adaptive Radiations

Striking adaptive radiations have also occurred over more limited geographic areas. Such radiations can be initiated

when a few organisms make their way to a new, often distant location in which they face relatively little competition from other organisms. The Hawaiian archipelago is one of the world's great showcases of this type of adaptive radiation (**Figure 25.22**). Located about 3,500 km from the nearest continent, the volcanic islands are progressively older as one follows the chain toward the northwest; the youngest



▲ **Figure 25.22 Adaptive radiation on the Hawaiian Islands.** Molecular analysis indicates that these remarkably varied Hawaiian plants, known collectively as the “silversword alliance,” are all descended from an ancestral tarweed that arrived on the islands from North America about 5 million years ago. Silverswords have since spread into different habitats and formed new species with strikingly different adaptations.

island, Hawaii, is less than a million years old and still has active volcanoes. Each island was born “naked” and was gradually populated by stray organisms that rode the ocean currents and winds either from far-distant land areas or from older islands of the archipelago itself. The physical diversity of each island, including immense variation in soil conditions, elevation, and rainfall, provides many opportunities for evolutionary divergence by natural selection. Multiple invasions followed by speciation events have ignited an explosion of adaptive radiation in Hawaii. As a result, thousands of species that inhabit the islands are found nowhere else on Earth. Among plants, for example, about 1,100 species are unique to the Hawaiian Islands. Unfortunately, many of these species are now facing an elevated risk of extinction due to human actions such as habitat destruction and the introduction of non-native plant species.



Animation: Overview of Macroevolution

CONCEPT CHECK 25.4

1. Explain the consequences of plate tectonics for life on Earth.
2. What factors promote adaptive radiations?
3. **WHAT IF? >** Suppose that an invertebrate species was lost in a mass extinction caused by a sudden catastrophic event. Would the last appearance of this species in the fossil record necessarily be close to when the extinction actually occurred? Would the answer to this question differ depending on whether the species was common (abundant and widespread) or rare? Explain.

For suggested answers, see Appendix A.

CONCEPT 25.5

Major changes in body form can result from changes in the sequences and regulation of developmental genes

The fossil record tells us what the great changes in the history of life have been and when they occurred. Moreover, an understanding of plate tectonics, mass extinction, and adaptive radiation provides a picture of how those changes came about. But we can also seek to understand the intrinsic biological mechanisms that underlie changes seen in the fossil record. For this, we turn to genetic mechanisms of change, paying particular attention to genes that influence development.

Effects of Developmental Genes

As you read in Concept 20.6, “evo-devo”—research at the interface between evolutionary biology and developmental biology—is illuminating how slight genetic differences can produce major morphological differences between species. In particular, large morphological differences can result from genes that alter the rate, timing, and spatial pattern of

change in an organism’s form as it develops from a zygote into an adult.

Changes in Rate and Timing

Many striking evolutionary transformations are the result of **heterochrony** (from the Greek *hetero*, different, and *chronos*, time), an evolutionary change in the rate or timing of developmental events. For example, an organism’s shape depends in part on the relative growth rates of different body parts during development. Changes to these rates can alter the adult form substantially, as seen in the contrasting shapes of human and chimpanzee skulls (**Figure 25.23**). Other examples of the dramatic evolutionary effects of heterochrony include how increased growth rates of finger bones yielded the skeletal structure of wings in bats (see Figure 21.15) and how slowed growth of leg and pelvic bones led to the reduction and eventual loss of hind limbs in whales (see Figure 21.20).

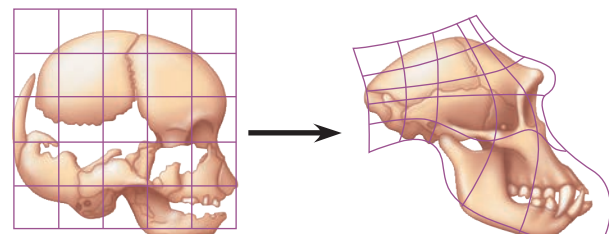
Heterochrony can also alter the timing of reproductive development relative to the development of nonreproductive organs. If the development of reproductive organs accelerates compared to that of other organs, the sexually mature stage of

▼ Figure 25.23 Relative skull growth rates. In the human evolutionary lineage, mutations slowed the growth of the jaw relative to other parts of the skull. As a result, in humans the skull of an adult is more similar to the skull of an infant than is the case for chimpanzees.



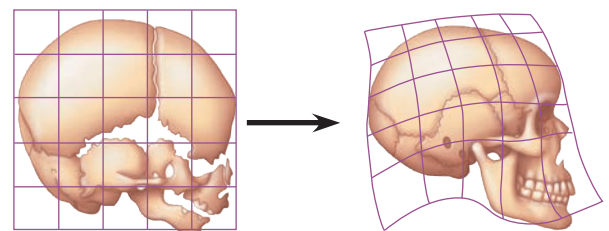
Chimpanzee infant

Chimpanzee adult



Chimpanzee fetus

Chimpanzee adult



Human fetus

Human adult



▲ Figure 25.24 Paedomorphosis. The adults of some species retain features that were juvenile in ancestors. This salamander is an axolotl, an aquatic species becomes a sexually mature adult while retaining certain larval (tadpole) characteristics, including gills.

a species may retain body features that were juvenile structures in an ancestral species, a condition called **paedomorphosis** (from the Greek *paedos*, of a child, and *morphosis*, formation). For example, most salamander species have aquatic larvae that undergo metamorphosis in becoming adults. But some species grow to adult size and become sexually mature while retaining gills and other larval features (Figure 25.24). Such an evolutionary alteration of developmental timing can produce animals that appear very different from their ancestors, even though the overall genetic change may be small. Indeed, recent evidence indicates that a change at a single locus was probably sufficient to bring about paedomorphosis in the axolotl salamander, although other genes may have contributed as well.

Changes in Spatial Pattern

Substantial evolutionary changes can also result from alterations in genes that control the spatial organization of body parts. For example, master regulatory genes called **homeotic genes** (see Concept 18.1) determine such basic features as where a pair of wings and legs will develop on a bird or how a plant's flower parts are arranged.

The products of one class of homeotic genes, the *Hox* genes, provide positional information in an animal embryo. This information prompts cells to develop into structures appropriate for a particular location. Changes in *Hox* genes or in how they are expressed can have a profound impact on morphology. For example, among crustaceans, a change in the location where two *Hox* genes (*Ubx* and *Scr*) are expressed correlates with the conversion of a swimming appendage to a feeding appendage. Similarly, when comparing plant species, changes to the expression of homeotic genes known as *MADS-box* genes can produce flowers that differ dramatically in form (see Concept 35.5).

The Evolution of Development

The 560-million-year-old fossils of Ediacaran animals in Figure 25.5 suggest that a set of genes sufficient to produce complex animals existed at least 25 million years *before* the Cambrian explosion. If such genes have existed for so long, how can we explain the astonishing increases in diversity seen during and since the Cambrian explosion?

Adaptive evolution by natural selection provides one answer to this question. As we've seen throughout this unit, by sorting among differences in the sequences of protein-encoding genes, selection can improve adaptations rapidly. In addition, new genes (created by gene duplication events) can take on new metabolic and structural functions, as can existing genes that are regulated in new ways.

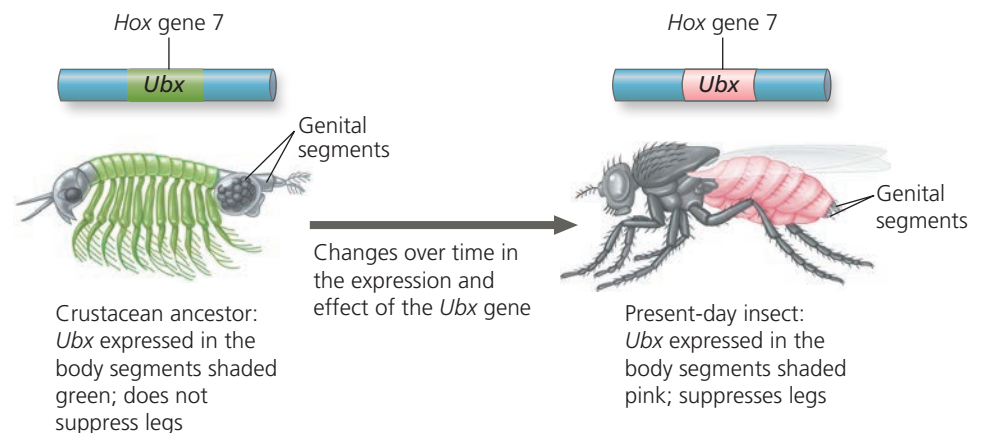
Examples in the previous section suggest that developmental genes may have been particularly important. Thus, we'll turn next to how new morphological forms can arise from changes in the nucleotide sequences or regulation of developmental genes.

Changes in Gene Sequence

New developmental genes arising after gene duplication events probably facilitated the origin of novel morphological forms. But since other genetic changes also may have occurred at such times, it can be difficult to establish causal links between genetic and morphological changes that occurred in the past.

This difficulty was sidestepped in a study of developmental changes associated with the divergence of six-legged insects from crustacean ancestors that had more than six legs. (As discussed in Concept 33.4, insects arose from within a subgroup of the crustaceans, the traditional name for organisms such as shrimp, crabs, and lobsters.) Researchers noted differences between crustaceans and insects in the pattern of expression and the effects of the *Hox* gene *Ubx*: In particular, in insects, *Ubx* suppresses leg formation where it is expressed (Figure 25.25).

▼ Figure 25.25 Effects of the *Hox* gene *Ubx* on the insect body plan. In crustaceans, the *Hox* gene *Ubx* is expressed in the region shaded green, the body segments between the head and genital segments. In insects, *Ubx* is expressed in only a subset (shaded pink) of the homologous body segments, where it suppresses leg formation.



To examine the workings of this gene, researchers cloned the *Ubx* gene from an insect, the fruit fly *Drosophila*, and from a crustacean, the brine shrimp *Artemia*. Next, they genetically engineered fruit fly embryos to express either the *Drosophila Ubx* gene or the *Artemia Ubx* gene throughout their bodies. The *Drosophila Ubx* gene suppressed 100% of the limbs in the embryos, as expected, whereas the *Artemia Ubx* gene suppressed only 15%.

The researchers then sought to uncover key steps involved in the evolutionary transition from an ancestral *Ubx* gene to an insect *Ubx* gene. Their approach was to identify mutations that would cause the *Artemia Ubx* gene to suppress leg formation, thus making its gene act more like an insect *Ubx* gene. To do this, they constructed a series of “hybrid” *Ubx* genes, each of which contained known segments of the *Drosophila Ubx* gene and known segments of the *Artemia Ubx* gene. By inserting these hybrid genes into fruit fly embryos (one hybrid gene per embryo) and observing their effects on leg development, the researchers were able to pinpoint the exact amino acid changes responsible for the suppression of additional limbs in insects. In so doing, this study provided evidence that particular changes in the nucleotide sequence of a developmental gene contributed to a major evolutionary change: the origin of the six-legged insect body plan.

Changes in Gene Regulation

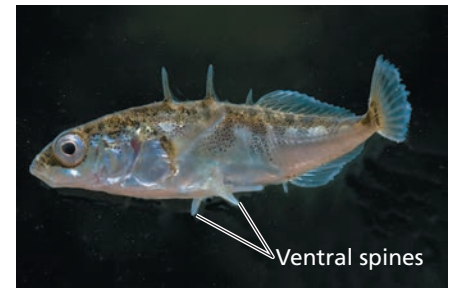
A change in the nucleotide sequence of a gene may affect its function wherever the gene is expressed, while changes in the regulation of gene expression can be limited to one cell type (see Concept 18.4). Thus, a change in the regulation of a developmental gene may have fewer harmful side effects than a change to the sequence of the gene. This reasoning has prompted researchers to suggest that changes in the form of organisms may often be caused by mutations that affect the regulation of developmental genes—not their sequences.

This idea is supported by studies of a variety of species, including threespine stickleback fish. These fish live in the open

▼ Figure 25.26

Inquiry What causes the loss of spines in lake stickleback fish?

Experiment Marine populations of the threespine stickleback fish (*Gasterosteus aculeatus*) have a set of protective spines on their lower (ventral) surface; however, these spines have been lost or reduced in some lake populations of this fish. Working at Stanford University, Michael Shapiro, David Kingsley, and colleagues performed genetic crosses and found that most of the reduction in spine size resulted from the effects of a single developmental gene, *Pitx1*. The researchers then tested two hypotheses about how *Pitx1* causes this morphological change.



▲ Threespine stickleback (*Gasterosteus aculeatus*)

Hypothesis A: A change in the DNA sequence of *Pitx1* had caused spine reduction in lake populations. To test this idea, the team used DNA sequencing to compare the coding sequence of the *Pitx1* gene between marine and lake stickleback populations.

Hypothesis B: A change in the regulation of the expression of *Pitx1* had caused spine reduction. To test this idea, the researchers monitored where in the developing embryo the *Pitx1* gene was expressed. They conducted whole-body *in situ* hybridization experiments (see Concept 19.2) using *Pitx1* DNA as a probe to detect *Pitx1* mRNA in the fish.

Results

Test of Hypothesis A: Are there differences in the coding sequence of the *Pitx1* gene in marine and lake stickleback fish?

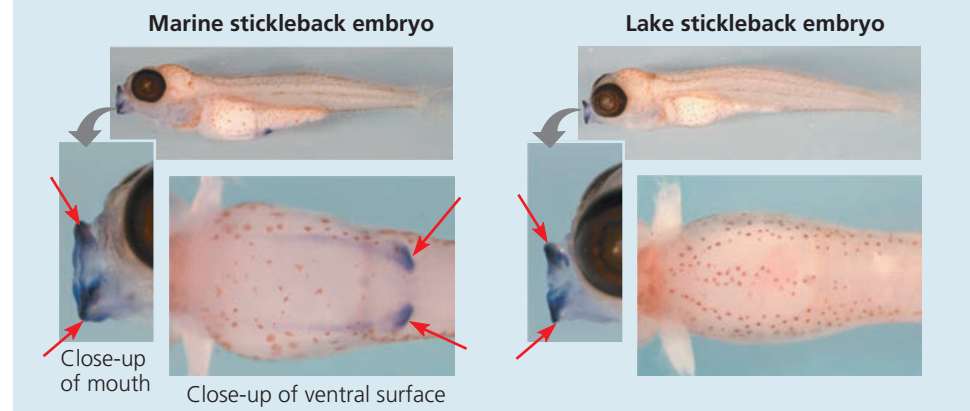
Result:
No

The 283 amino acids of the *Pitx1* protein are identical in marine and lake stickleback populations.

Test of Hypothesis B: Are there any differences in the regulation of expression of *Pitx1*?

Result:
Yes

Red arrows (→) indicate regions of *Pitx1* gene expression in the photographs below. *Pitx1* is expressed in the ventral spine and mouth regions of developing marine stickleback fish but only in the mouth region of developing lake stickleback fish.



Conclusion The loss or reduction of ventral spines in lake populations of threespine stickleback fish appears to have resulted primarily from a change in the regulation of *Pitx1* gene expression, not from a change in the gene's sequence.

Data from M. D. Shapiro et al., Genetic and developmental basis of evolutionary pelvic reduction in three-spine sticklebacks, *Nature* 428:717–723 (2004).

WHAT IF? > Describe the set of results that would have led researchers to the conclusion that a change in the coding sequence of the *Pitx1* gene was more important than a change in regulation of gene expression.

ocean and in shallow, coastal waters. In western Canada, they also live in lakes formed when the coastline receded during the past 12,000 years. Marine stickleback fish have a pair of spines on their ventral (lower) surface, which deter some predators. These spines are often reduced or absent in stickleback fish living in lakes that lack predatory fishes and that are also low in calcium. Spines may have been lost in such lakes because they are not advantageous in the absence of predators, and the limited calcium is needed for purposes other than constructing spines.

At the genetic level, the developmental gene *Pitx1* was known to influence whether stickleback fish have ventral spines. Was the reduction of spines in some lake populations due to changes in the *Pitx1* gene or to changes in how the gene is expressed (Figure 25.26)? The researchers' results indicate that the regulation of gene expression has changed, not the DNA sequence. Moreover, lake stickleback fish do express the *Pitx1* gene in tissues not related to the production of spines (for example, the mouth), illustrating how morphological change can be caused by altering the expression of a developmental gene in some parts of the body but not others. In a 2010 follow-up study, researchers showed that changes to the *Pel* enhancer, a noncoding DNA region that affects expression of the *Pitx1* gene, resulted in the reduction of ventral spines in lake sticklebacks. Overall, results from studies on stickleback fish provide a clear and detailed example of how changes in gene regulation can alter the form of individual organisms and ultimately lead to evolutionary change in populations.



HHMI Video: The Making of the Fittest: Evolving Switches, Evolving Bodies (Stickleback)



CONCEPT CHECK 25.5

1. Explain how new body forms can originate by heterochrony.
2. Why is it likely that *Hox* genes have played a major role in the evolution of novel morphological forms?
3. **MAKE CONNECTIONS** ▶ Given that changes in morphology are often caused by changes in the regulation of gene expression, predict whether noncoding DNA is likely to be affected by natural selection. See Concept 18.3 to review noncoding DNA and regulation of gene expression.

For suggested answers, see Appendix A.

CONCEPT 25.6

Evolution is not goal oriented

What does our study of macroevolution tell us about how evolution works? One lesson is that throughout the history of life, the origin of new species has been affected by both the small-scale factors described in Concept 23.3 (such as natural selection operating in populations) and the large-scale factors described in this chapter (such as continental drift promoting bursts of speciation throughout the globe). Moreover, to paraphrase the Nobel Prize-winning geneticist François Jacob, evolution is like tinkering—a process in which new forms arise by the modification of existing structures or existing

developmental genes. Over time, such tinkering has led to the three key features of the natural world described on the opening page of Chapter 21: the striking ways in which organisms are suited for life in their environments, the many shared characteristics of life, and the rich diversity of life.

Evolutionary Novelties

François Jacob's view of evolution harkens back to Darwin's concept of descent with modification. As new species form, novel and complex structures can arise as gradual modifications of ancestral structures. In many cases, complex structures have evolved in increments from simpler versions that performed the same basic function. For example, consider the human eye, an intricate organ constructed from numerous parts that work together in forming an image and transmitting it to the brain. How could the human eye have evolved in gradual increments? Some argue that if the eye needs all of its components to function, a partial eye could not have been of use to our ancestors.

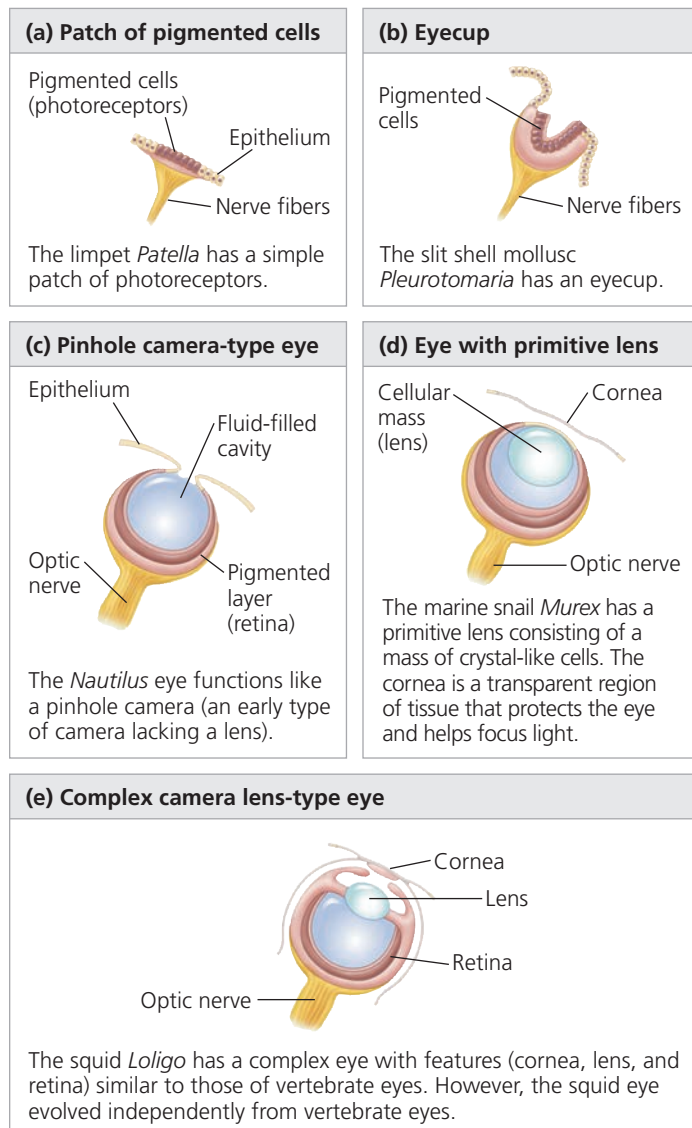
The flaw in this argument, as Darwin himself noted, lies in the assumption that only complicated eyes are useful. In fact, many animals depend on eyes that are far less complex than our own. The simplest eyes that we know of are patches of light-sensitive photoreceptor cells. These simple eyes appear to have had a single evolutionary origin and are now found in a variety of animals, including small molluscs called limpets. Such eyes have no equipment for focusing images, but they do enable the animal to distinguish light from dark. Limpets cling more tightly to their rock when a shadow falls on them, a behavioral adaptation that reduces the risk of being eaten (Figure 25.27). Limpets have had a long evolutionary history, demonstrating that their “simple” eyes are quite adequate to support their survival and reproduction.

In the animal kingdom, complex eyes have evolved independently from such basic structures many times. Some molluscs, such as squids and octopuses, have eyes as complex as those

▼ **Figure 25.27 Limpets (*Patella vulgata*), molluscs that can sense light and dark with a simple patch of photoreceptor cells.**



▼ **Figure 25.28 A range of eye complexity among molluscs.**



of humans and other vertebrates (Figure 25.28). Although complex mollusc eyes evolved independently of vertebrate eyes, both evolved from a simple cluster of photoreceptor cells present in a common ancestor. In each case, the complex eye evolved through a series of steps that benefited the eyes' owners at every stage. Evidence of their independent evolution can be found in their structure: Vertebrate eyes detect light at the back layer of the retina and conduct nerve impulses toward the front, while complex mollusc eyes do the reverse.

Throughout their evolutionary history, eyes retained their basic function of vision. But evolutionary novelties can also arise when structures that originally played one role gradually acquire a different one. For example, as cynodonts gave rise to early mammals, bones that formerly comprised the jaw hinge (the articular and quadrate; see Figure 25.7) were incorporated into the ear region of mammals, where they eventually took on a new function: the transmission of sound

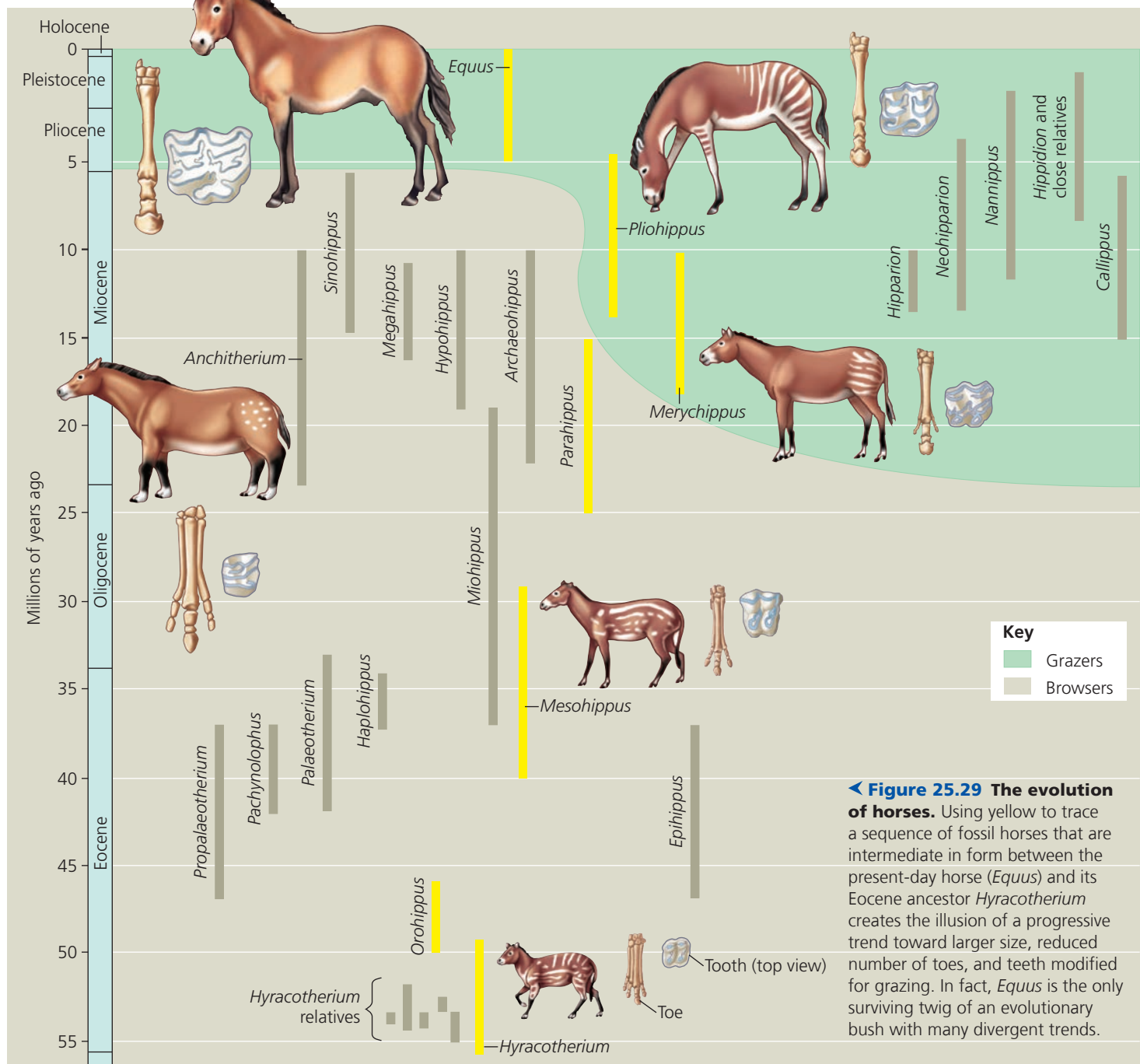
(see Concept 34.6). Structures that evolve in one context but become co-opted for another function are sometimes called *exaptations* to distinguish them from the adaptive origin of the original structure. Note that the concept of exaptation does not imply that a structure somehow evolves in anticipation of future use. Natural selection cannot predict the future; it can only improve a structure in the context of its *current* utility. Novel features, such as the new jaw hinge and ear bones of early mammals, can arise gradually via a series of intermediate stages, each of which has some function in the organism's current context.

Evolutionary Trends

What else can we learn from patterns of macroevolution? Consider evolutionary "trends" observed in the fossil record. For instance, some evolutionary lineages exhibit a trend toward larger or smaller body size. An example is the evolution of the present-day horse (genus *Equus*), a descendant of the 55-million-year-old *Hyracotherium* (Figure 25.29). About the size of a large dog, *Hyracotherium* had four toes on its front feet, three toes on its hind feet, and teeth adapted for browsing on bushes and trees. In comparison, present-day horses are larger, have only one toe on each foot, and possess teeth modified for grazing on grasses.

Extracting a single evolutionary progression from the fossil record can be misleading, however; it is like describing a bush as growing toward a single point by tracing only the branches that lead to that twig. For example, by selecting certain species from the available fossils, it is possible to arrange a succession of animals intermediate between *Hyracotherium* and living horses that shows a trend toward large, single-toed species (follow the yellow highlighting in Figure 25.29). However, if we consider *all* fossil horses known today, this apparent trend vanishes. The genus *Equus* did not evolve in a straight line; it is the only surviving twig of an evolutionary tree that is so branched that it is more like a bush. *Equus* actually descended through a series of speciation episodes that included several adaptive radiations, not all of which led to large, one-toed, grazing horses. In fact, phylogenetic analyses suggest that all lineages that include grazers are closely related to *Parahippus*; the many other horse lineages, all of which are now extinct, remained multi-toed browsers for 35 million years.

Branching evolution *can* result in a real evolutionary trend even if some species counter the trend. One model of long-term trends views species as analogous to individuals: Speciation is their birth, extinction is their death, and new species that diverge from them are their offspring. In this model, just as populations of individual organisms undergo natural selection, species undergo *species selection*. The species that endure the longest and generate the most new offspring species determine the direction of major evolutionary trends. The species selection model suggests that "differential speciation success" plays a role in macroevolution similar to the role of differential reproductive success in microevolution. Evolutionary trends can



also result directly from natural selection. For example, when horse ancestors invaded the grasslands that spread during the mid-Cenozoic, there was strong selection for grazers that could escape predators by running faster. This trend would not have occurred without open grasslands.

Whatever its cause, an evolutionary trend does not imply that there is some intrinsic drive toward a particular phenotype. Evolution is the result of the interactions between organisms and their current environments; if environmental conditions change, an evolutionary trend may cease or even reverse itself. The cumulative effect of these ongoing interactions between organisms and their environments is enormous: It is through them that the

staggering diversity of life—Darwin’s “endless forms most beautiful”—has arisen.

CONCEPT CHECK 25.6

- How can the Darwinian concept of descent with modification explain the evolution of such complex structures as the vertebrate eye?
- WHAT IF? >** The myxoma virus kills up to 99.8% of infected European rabbits in populations with no previous exposure to the virus. The virus is transmitted between living rabbits by mosquitoes. Describe an evolutionary trend (in either the rabbit or virus) that might occur after a rabbit population first encounters the virus.

For suggested answers, see Appendix A.

25 Chapter Review



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SUMMARY OF KEY CONCEPTS

CONCEPT 25.1

Conditions on early Earth made the origin of life possible (pp. 580–582)

- Experiments simulating possible early atmospheres have produced organic molecules from inorganic precursors. Amino acids, lipids, sugars, and nitrogenous bases have also been found in meteorites.
- Amino acids and RNA nucleotides polymerize when dripped onto hot sand, clay, or rock. Organic compounds can spontaneously assemble into **protocells**, membrane-bounded droplets that have some properties of cells.
- The first genetic material may have self-replicating, catalytic RNA. Early protocells containing such RNA would have increased through natural selection.

? Describe the roles that montmorillonite clay and vesicles may have played in the origin of life.



VOCAB
SELF-QUIZ
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CONCEPT 25.2

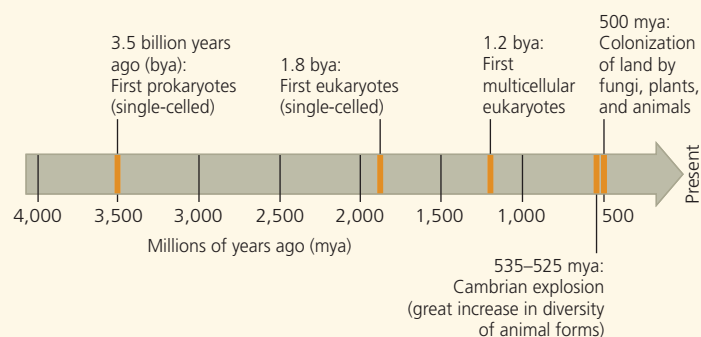
The fossil record documents the history of life (pp. 582–586)

- The fossil record, based largely on fossils found in sedimentary rocks, documents the rise and fall of different groups of organisms over time.
- Sedimentary strata reveal the relative ages of fossils. The ages of fossils can be estimated by **radiometric dating** and other methods.
- The fossil record shows how new groups of organisms can arise via the gradual modification of preexisting organisms.

? What are the challenges of estimating the ages of old fossils? Explain how these challenges may be overcome in some circumstances.

CONCEPT 25.3

Key events in life's history include the origins of unicellular and multicellular organisms and the colonization of land (pp. 586–591)



? What is the “Cambrian explosion,” and why is it significant?

CONCEPT 25.4

The rise and fall of groups of organisms reflect differences in speciation and extinction rates (pp. 591–598)

- In **plate tectonics**, continental plates move gradually over time, altering the physical geography and climate of Earth, leading to extinctions in some groups and speciation in others.
- Evolutionary history has been punctuated by five **mass extinctions** that radically altered life's history. Possible causes for these extinctions include continental drift, volcanic activity, and impacts from comets.
- Large increases in the diversity of life have resulted from **adaptive radiations** that followed mass extinctions. Adaptive radiations have also occurred in groups of organisms that possessed major evolutionary innovations or that colonized new regions in which there was little competition from other organisms.

? Explain how the broad evolutionary changes seen in the fossil record are the cumulative result of speciation and extinction events.

CONCEPT 25.5

Major changes in body form can result from changes in the sequences and regulation of developmental genes (pp. 598–601)

- Developmental genes affect morphological differences between species by influencing the rate, timing, and spatial patterns of change in an organism's form as it develops into an adult.
- The evolution of new forms can be caused by changes in the nucleotide sequences or regulation of developmental genes.

? How could changes in a single gene or DNA region ultimately lead to the origin of a new group of organisms?

CONCEPT 25.6

Evolution is not goal oriented (pp. 601–603)

- Novel and complex biological structures can evolve through a series of incremental modifications, each of which benefits the organism that possesses it.
- Evolutionary trends can be caused by natural selection in a changing environment or species selection, resulting from interactions between organisms and their current environments.

? Explain the reasoning behind the statement “Evolution is not goal oriented.”

TEST YOUR UNDERSTANDING



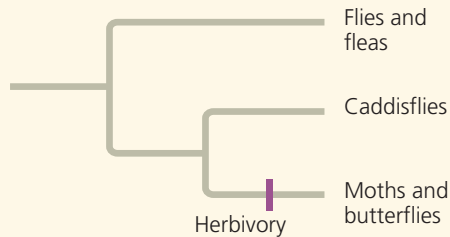
Multiple-choice Self-Quiz questions 1–7 can be found in the Study Area in MasteringBiology.

8. **EVOLUTION CONNECTION** Describe how gene flow, genetic drift, and natural selection all can influence macroevolution.



PRACTICE
TEST
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- 9. SCIENTIFIC INQUIRY** Herbivory (plant eating) has evolved repeatedly in insects, typically from meat-eating or detritus-feeding ancestors (detritus is dead organic matter). Moths and butterflies, for example, eat plants, whereas their “sister group” (the insect group to which they are most closely related), the caddisflies, feed on animals, fungi, or detritus. As illustrated in the following phylogenetic tree, the combined moth/butterfly and caddisfly group shares a common ancestor with flies and fleas. Like caddisflies, flies and fleas are thought to have evolved from ancestors that did not eat plants.



There are 140,000 species of moths and butterflies and 7,000 species of caddisflies. State a hypothesis about the impact of herbivory on adaptive radiations in insects. How could this hypothesis be tested?

- 10. WRITE ABOUT A THEME: ORGANIZATION** You have seen many examples of how form fits function at all levels of the biological hierarchy. However, we can imagine forms that would function better than some forms actually found in nature. For example, if the wings of a bird were not formed from its forelimbs, such a hypothetical bird could fly yet also hold objects with its forelimbs. In a short essay (100–150 words), use the concept of “evolution as tinkering” to explain why there are limits to the functionality of forms in nature.

11. SYNTHESIZE YOUR KNOWLEDGE



In 2010, the Soufriere Hills volcano on the Caribbean island of Montserrat erupted violently, spewing huge clouds of ash and gases into the sky. Explain how the volcanic eruptions at the end of the Permian period and the formation of Pangaea, both of which occurred about 252 million years ago, set in motion events that altered evolutionary history.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

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UNIT 5

THE DIVERSITY OF LIFE

Winner of a MacArthur Fellowship “Genius” Award, Nancy A. Moran is the Leslie Surginer Endowed Professor of Integrative Biology at the University of Texas at Austin. Dr. Moran received a B.A. in general studies from the University of Texas, graduating with highest honors, and a Ph.D. in zoology from the University of Michigan. Elected to the U.S. National Academy of Sciences in 2004, Dr. Moran has a long-standing interest in the evolutionary history and ecological importance of symbioses. Currently, she and her students are using experimental and phylogenomic approaches to study beneficial interactions between insects such as aphids and honeybees and bacteria that live within their bodies.



An Interview with Nancy Moran

How did you first become interested in science?

As a kid, I liked insects and enjoyed gardening. However, I did not see myself as becoming a scientist, and I began college as an art major. But for me, art was just too hard, and I switched into an honors program that did not require you to declare a major. As a junior, I happened to take a class in biology, which I really enjoyed. So I took more biology courses and eventually did an honors thesis on mate choice in birds. By then, I was hooked!

Much of your work is on symbioses. What are symbioses, and what drew you to this topic?

A symbiosis is a close physiological relationship in which one species (the symbiont) lives in or on a larger species (the host). In the late 1980s, I was working on aphids, which have many bacterial symbionts. No one knew what these symbionts did because they can't live outside of their host. I wasn't planning to study symbioses, but then I got an intriguing phone call from the bacteriologist Paul Baumann. At that time, PCR had just become widely available, which enabled researchers to amplify and sequence particular genes. Paul had the foresight to realize that we could use PCR to ask, “What kinds of bacteria live within aphids as symbionts, and based on their genes, what are these symbionts doing?”

“We sequenced these genes and determined that they came from a fungus. What a surprise—the carotenoid genes of a fungus had become part of the DNA of an aphid!”

▼ **Adult female pea aphids and asexually produced daughters. The reddish color is due to carotenoid pigments.**



How do symbioses affect life on Earth?

Symbioses are everywhere. Every organism is engaged in symbioses, including humans. As one example, our bodies contain bacteria that break down carbohydrates that we otherwise could not digest. In the case of aphids, these insects feed on plant sap, which provides sugars but lacks other essential nutrients, such as key amino acids. These amino acids are supplied to the aphids by bacterial symbionts. At a higher level, we can say that entire ecosystems depend on symbioses. Coral reefs are built by coral polyps that depend on energy provided by algal symbionts. Without their symbionts, the corals can die, harming the reef and the many fishes and other organisms that live there.

What is the most surprising discovery that you've made?

One such discovery concerns carotenoids, which are colored molecules used in photosynthesis by plants and in light detection by many animals. It was thought that animals could not make carotenoids and so had to get them from their diet. While studying the genome of an aphid, we were startled to find that it had functional carotenoid-synthesizing genes. We sequenced these genes and determined that they came from a fungus. What a surprise—the carotenoid genes of a fungus had become part of the DNA of an aphid!

What advice do you have for students considering a career in biology?

Every student is different. Some students categorize themselves as not interested in or unable to “do” science. But the only way to tell is to try it: Dive in, see if you enjoy research, and discover the special skills that you bring to the table. Biology needs people with different skills. One person might excel at communication, another is meticulous, while a third can synthesize many different pieces of information. If you find that you enjoy biology, go for it—whether you are a freshman or a senior, it is not too late.

Introduction to Viruses

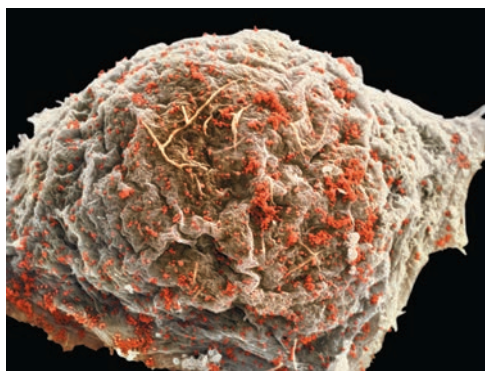
26



▲ **Figure 26.1** Are the viruses (reddish-purple) budding from these cells alive?

KEY CONCEPTS

- 26.1** A virus consists of a nucleic acid surrounded by a protein coat
- 26.2** Viruses replicate only in host cells
- 26.3** Viruses and prions are formidable pathogens in animals and plants



▲ A human immune cell infected with HIV. New viruses (red) are budding from the plasma membrane (colorized SEM).

A Borrowed Life

The illustration in **Figure 26.1** shows a remarkable event: Human immune cells (purple) infected by human immunodeficiency viruses (HIV) are releasing more HIV viruses. These viruses (red, surrounded by a protein-studded purple membrane from the immune cell) will infect other cells. (The SEM below shows one infected cell.) By injecting its genetic information into a cell, a virus hijacks the cell, recruiting cellular machinery to manufacture many new viruses and promote further infection. Left untreated, HIV causes acquired immunodeficiency syndrome (AIDS) by destroying vital immune system cells.

Compared with eukaryotic and even prokaryotic cells, viruses are much smaller and simpler in structure. Lacking the structures and metabolic machinery found in a cell, a **virus** is an infectious particle consisting of little more than genes packaged in a protein coat.

Are viruses living or nonliving? Early on, they were considered biological chemicals; the Latin root for virus means “poison.” Viruses are capable of causing a wide variety of diseases, so researchers in the late 1800s saw a parallel with bacteria and proposed that viruses were the simplest of living forms. However, viruses cannot reproduce or carry out metabolic activities outside of a host cell. Most biologists studying viruses today would probably agree that they are not alive but exist in a shady area between life-forms and chemicals. The simple phrase used recently by two researchers describes them aptly enough: Viruses lead “a kind of borrowed life.”

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

To a large extent, molecular biology was born in the laboratories of biologists studying viruses that infect bacteria. Experiments with these viruses provided evidence that genes are made of nucleic acids, and they were critical in working out the molecular mechanisms of the fundamental processes of DNA replication, transcription, and translation.

In this chapter, we will explore the biology of viruses, beginning with their structure and then describing how they replicate. Next, we will discuss the role of viruses as disease-causing agents, or pathogens, and conclude by considering some even simpler infectious agents called prions.

CONCEPT 26.1

A virus consists of a nucleic acid surrounded by a protein coat

Scientists were able to detect viruses indirectly long before they were actually able to see them. The story of how viruses were discovered begins near the end of the 19th century.

The Discovery of Viruses: *Scientific Inquiry*

Tobacco mosaic disease stunts the growth of tobacco plants and gives their leaves a mottled, or mosaic, coloration. In 1883, Adolf Mayer, a German scientist, discovered that he could transmit the disease from plant to plant by rubbing sap extracted from diseased leaves onto healthy plants. After an unsuccessful search for an infectious microbe in the sap, Mayer suggested that the disease was caused by unusually small bacteria that were invisible under a microscope. This hypothesis was tested a decade later by Dmitri Ivanowsky, a Russian biologist who passed sap from infected tobacco leaves through a filter designed to remove bacteria. After filtration, the sap still produced mosaic disease.

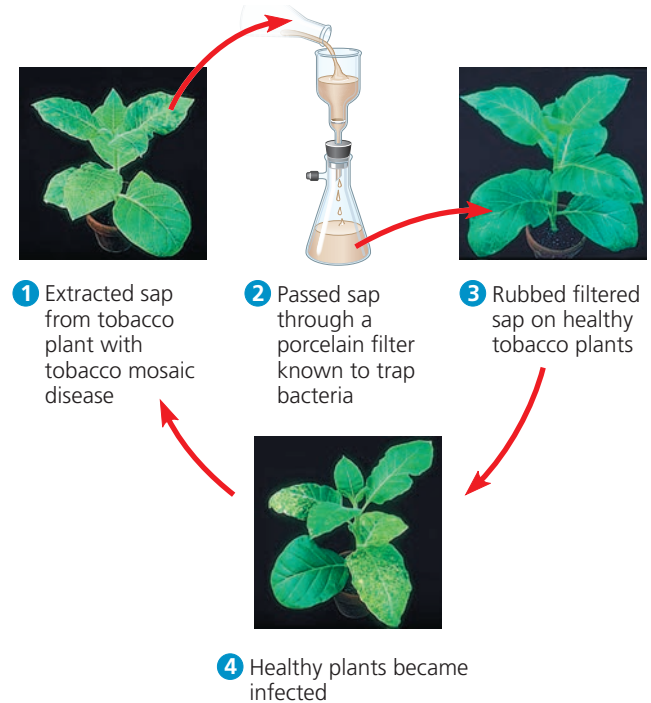
But Ivanowsky clung to the hypothesis that bacteria caused tobacco mosaic disease. Perhaps, he reasoned, the bacteria were small enough to pass through the filter or made a toxin that could do so. The second possibility was ruled out when the Dutch botanist Martinus Beijerinck carried out a classic series of experiments that showed that the infectious agent in the filtered sap could replicate (**Figure 26.2**).

In fact, the pathogen replicated only within the host it infected. In further experiments, Beijerinck showed that unlike bacteria used in the lab at that time, the mysterious agent of mosaic disease could not be cultivated on nutrient media in test tubes or petri dishes. Beijerinck imagined a replicating particle much smaller and simpler than a bacterium, and he is generally credited with being the first scientist to voice the concept of a virus. His suspicions were confirmed in 1935 when the American scientist Wendell Stanley crystallized the infectious particle, now known as tobacco mosaic virus (TMV). Subsequently, TMV and many other viruses were actually seen with the help of the electron microscope.

▼ Figure 26.2

Inquiry What causes tobacco mosaic disease?

Experiment In the late 1800s, Martinus Beijerinck, of the Technical School in Delft, the Netherlands, investigated the properties of the agent that causes tobacco mosaic disease (then called spot disease).



Results When the filtered sap was rubbed on healthy plants, they became infected. Their sap, extracted and filtered, could then act as a source of infection for another group of plants. Each successive group of plants developed the disease to the same extent as earlier groups.

Conclusion The infectious agent was apparently not a bacterium because it could pass through a bacterium-trapping filter. The pathogen must have been replicating in the plants because its ability to cause disease was undiluted after several transfers from plant to plant.

Data from M. J. Beijerinck, Concerning a *contagium vivum fluidum* as cause of the spot disease of tobacco leaves, *Verhandelingen der Koninklijke akademie Wetenschappen te Amsterdam* 65:3–21 (1898). Translation published in English as *Phytopathological Classics* Number 7 (1942), American Phytopathological Society Press, St. Paul, MN.

WHAT IF? > If Beijerinck had observed that the infection of each group was weaker than that of the previous group and that ultimately the sap could no longer cause disease, what might he have concluded?

Structure of Viruses

The tiniest viruses are only 20 nm in diameter—smaller than a ribosome. Millions could easily fit on a pinhead. Even the largest known virus, which has a diameter of 1,500 nanometers (1.5 μm), is barely visible under the light microscope. Stanley's discovery that some viruses could be crystallized was exciting and puzzling news. Not even the simplest of cells can aggregate into regular crystals. But if viruses are not cells, then what are they? Examining the structure of a virus more closely reveals that it is an infectious particle consisting of nucleic acid enclosed in a protein coat and, for some viruses, surrounded by a membranous envelope.

Viral Genomes

We usually think of genes as being made of double-stranded DNA, but many viruses defy this convention. Their genomes may consist of double-stranded DNA, single-stranded DNA, double-stranded RNA, or single-stranded RNA, depending on the type of virus. A virus is called a DNA virus or an RNA virus based on the kind of nucleic acid that makes up its genome. In either case, the genome is usually organized as a single linear or circular molecule of nucleic acid, although the genomes of some viruses consist of multiple molecules of nucleic acid. The smallest viruses known have only three genes in their genome, while the largest have several hundred to 2,000. For comparison, bacterial genomes contain about 200 to a few thousand genes.

Capsids and Envelopes

The protein shell enclosing the viral genome is called a **capsid**. Depending on the type of virus, the capsid may be rod-shaped, polyhedral, or more complex in shape. Capsids are built from a large number of protein subunits called

capsomeres, but the number of different *kinds* of proteins in a capsid is usually small. Tobacco mosaic virus has a rigid, rod-shaped capsid made from over 1,000 molecules of a single type of protein arranged in a helix; rod-shaped viruses are commonly called *helical viruses* for this reason (**Figure 26.3a**). Adenoviruses, which infect the respiratory tracts of animals, have 252 identical protein molecules arranged in a polyhedral capsid with 20 triangular facets—an icosahedron; thus, these and other similarly shaped viruses are referred to as *icosahedral viruses* (**Figure 26.3b**).

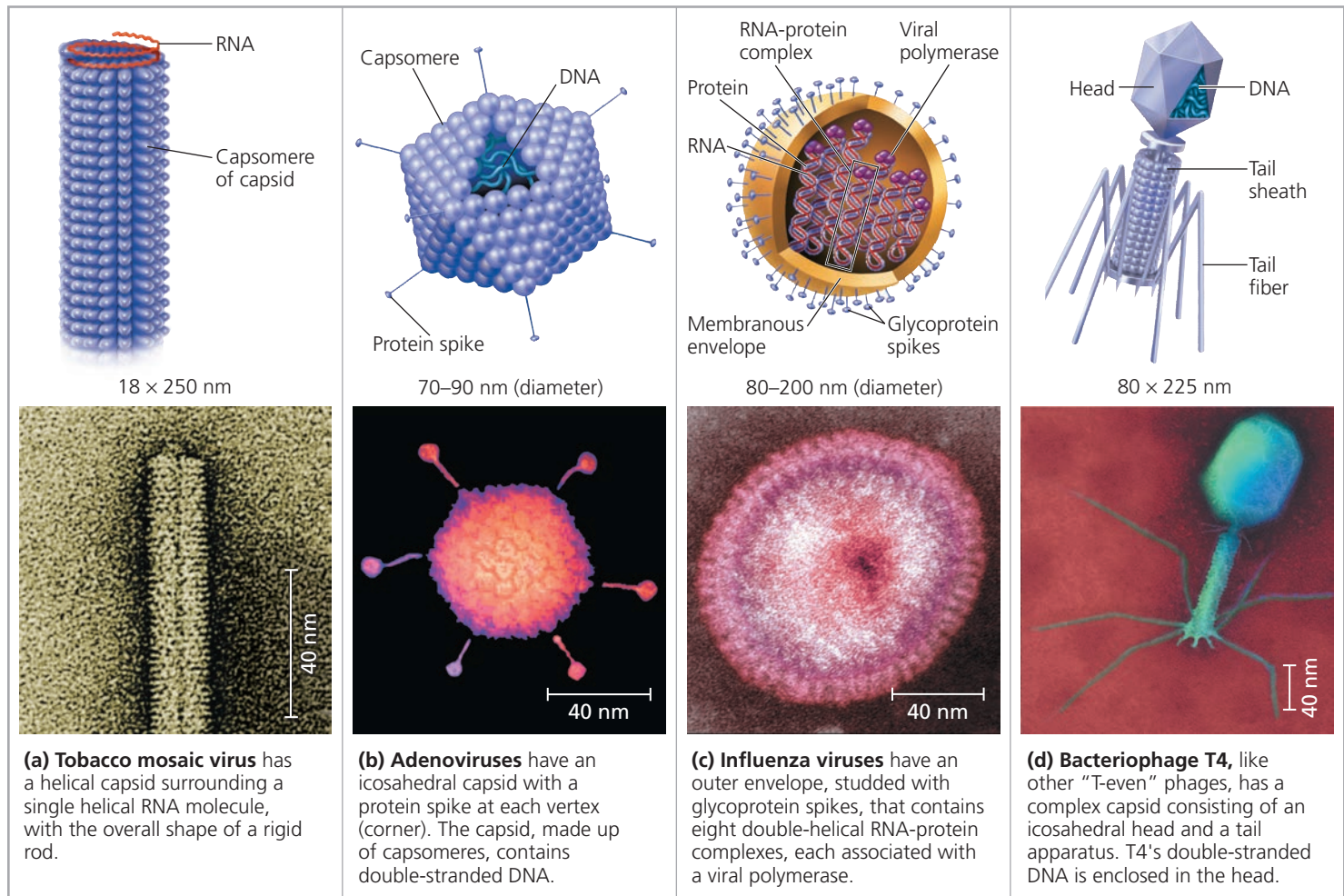
Some viruses have accessory structures that help them infect their hosts. For instance, a membranous envelope surrounds the capsids of influenza viruses and many other viruses found in animals (**Figure 26.3c**). These **viral envelopes**, which are derived from the membranes of the host cell, contain host cell phospholipids and membrane proteins. They also contain proteins and glycoproteins of viral origin. (Glycoproteins are proteins with carbohydrates covalently attached.) Some viruses carry a few viral enzyme molecules within their capsids.

Many of the most complex capsids are found among the viruses that infect bacteria, called **bacteriophages**,

▼ **Figure 26.3 Viral structure.** Viruses are made up of nucleic acid (DNA or RNA) enclosed in a protein coat (the capsid) and sometimes

further wrapped in a membranous envelope. The individual protein subunits making up the capsid are called capsomeres. Although

diverse in size and shape, viruses have many common structural features. (All micrographs are colorized TEMs.)



or simply **phages**. The first phages studied included seven that infect *Escherichia coli*. These seven phages were named type 1 (T1), type 2 (T2), and so forth, in the order of their discovery. The three “T-even” phages (T2, T4, and T6) turned out to be very similar in structure. Their capsids have elongated icosahedral heads enclosing their DNA. Attached to the head is a protein tail piece with fibers by which the phages attach to a bacterial cell (**Figure 26.3d**). In the next section, we’ll examine how these few viral parts function together with cellular components to produce large numbers of viral progeny.

CONCEPT CHECK 26.1

1. **VISUAL SKILLS** > Compare the structures of tobacco mosaic virus (TMV) and influenza virus (see Figure 26.3).
2. **MAKE CONNECTIONS** > Bacteriophages were used to provide evidence that DNA carries genetic information (see Figure 16.4). Briefly describe the experiment carried out by Hershey and Chase, including in your description why the researchers chose to use phages.

For suggested answers, see Appendix A.

CONCEPT 26.2

Viruses replicate only in host cells

Viruses lack metabolic enzymes and equipment for making proteins, such as ribosomes. They are obligate intracellular parasites; in other words, they can replicate only within a host cell. It is fair to say that viruses in isolation are merely packaged sets of genes in transit from one host cell to another.

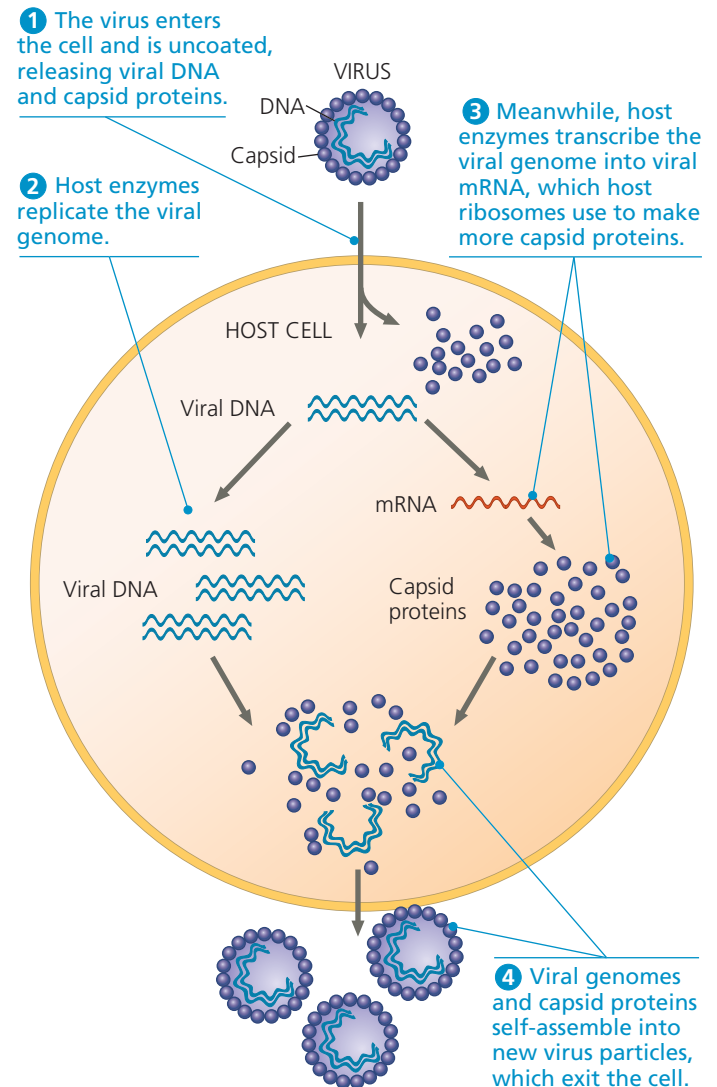
Each particular virus can infect cells of only a limited number of host species, called the **host range** of the virus. This host specificity results from the evolution of recognition systems by the virus. Viruses usually identify host cells by a “lock-and-key” fit between viral surface proteins and specific receptor molecules on the outside of cells. According to one model, such receptor molecules originally carried out functions that benefited the host cell but were co-opted later by viruses as portals of entry. Some viruses have broad host ranges. For example, West Nile virus and equine encephalitis virus are distinctly different viruses that can each infect mosquitoes, birds, horses, and humans. Other viruses have host ranges so narrow that they infect only a single species. Measles virus, for instance, can infect only humans. Furthermore, viral infection of multicellular eukaryotes is usually limited to particular tissues. Human cold viruses infect only the cells lining the upper respiratory tract, and the HIV seen in Figure 26.1 binds to receptors present only on certain types of immune cells.

General Features of Viral Replicative Cycles

A viral infection begins when a virus binds to a host cell and the viral genome makes its way inside (**Figure 26.4**). The mechanism of genome entry depends on the type of virus and the type of host cell. For example, T-even phages use their

elaborate tail apparatus to inject DNA into a bacterium (see Figure 26.3d). Other viruses are taken up by endocytosis or, in the case of enveloped viruses, by fusion of the viral envelope with the host’s plasma membrane. Once the viral genome is inside, the proteins it encodes can commandeer the host, reprogramming the cell to copy the viral genome and manufacture viral proteins. The host provides the nucleotides for making viral nucleic acids, as well as enzymes, ribosomes, tRNAs, amino acids, ATP, and other components needed for making the viral proteins. Many DNA viruses use the DNA polymerases of the host cell to synthesize new genomes along the templates provided by the viral DNA. In contrast, to replicate their genomes, RNA viruses use virally encoded RNA polymerases that can use RNA as a template. (Uninfected cells generally make no enzymes for carrying out this process.)

▼ **Figure 26.4 A simplified viral replicative cycle.** A virus is an intracellular parasite that uses the equipment and small molecules of its host cell to replicate. In this simplest of viral cycles, the parasite is a DNA virus with a capsid consisting of a single type of protein.



MAKE CONNECTIONS > Label each of the straight gray arrows with one word representing the name of the process that is occurring. Review Figure 17.25.

Animation: Simplified Viral Replicative Cycle

After the viral nucleic acid molecules and capsomeres are produced, they spontaneously self-assemble into new viruses. In fact, researchers can separate the RNA and capsomeres of TMV and then reassemble complete viruses simply by mixing the components together under the right conditions. The simplest type of viral replicative cycle ends with the exit of hundreds or thousands of viruses from the infected host cell, a process that often damages or destroys the cell. Such cellular damage and death, as well as the body's responses to this destruction, cause many of the symptoms associated with viral infections. The viral progeny that exit a cell have the potential to infect additional cells, spreading the viral infection.

There are many variations on the simplified viral replicative cycle we have just described. We will now take a look at some of these variations in bacterial viruses (phages) and animal viruses; later in the chapter, we will consider plant viruses.

Replicative Cycles of Phages

Phages are the best understood of all viruses, although some of them are also among the most complex. Research on phages led to the discovery that some double-stranded DNA viruses can replicate by two alternative mechanisms: the lytic cycle and the lysogenic cycle.

The Lytic Cycle

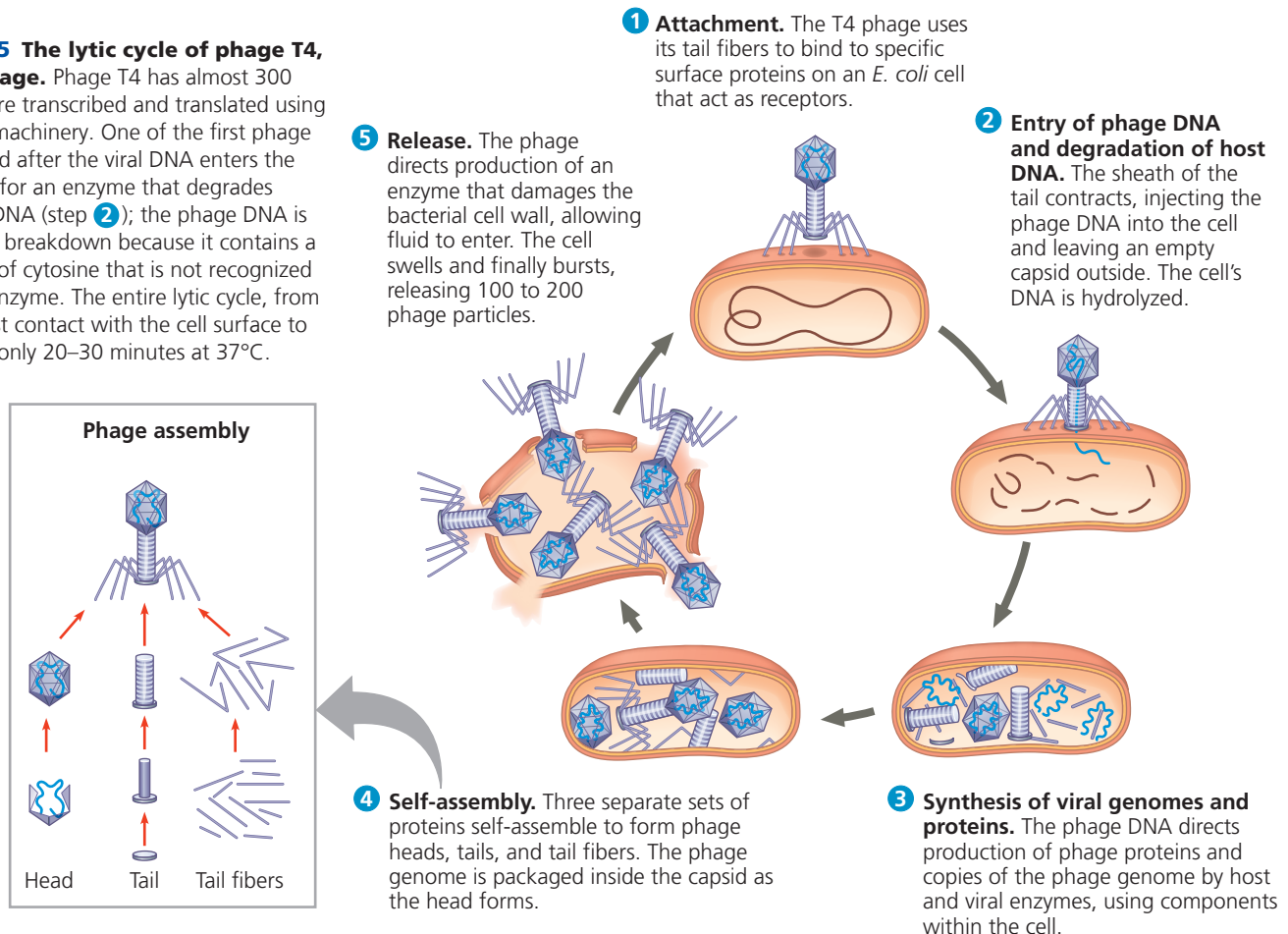
A phage replicative cycle that culminates in death of the host cell is known as a **lytic cycle**. The term refers to the last stage of infection, during which the bacterium lyses (breaks open) and releases the phages that were produced within the cell. Each of these phages can then infect a healthy cell, and a few successive lytic cycles can destroy an entire bacterial population in just a few hours. A phage that replicates only by a lytic cycle is a **virulent phage**. **Figure 26.5** illustrates the major steps in the lytic cycle of T4, a typical virulent phage.

The Lysogenic Cycle

Instead of lysing their host cells, many phages coexist with them in a state called lysogeny. In contrast to the lytic cycle, which kills the host cell, the **lysogenic cycle** allows replication of the phage genome without destroying the host. Phages capable of using both modes of replicating within a bacterium are called **temperate phages**. A temperate phage called lambda, written with the Greek letter λ , has been widely used in biological research. Phage λ resembles T4, but its tail has only one short tail fiber.

Infection of an *E. coli* cell by phage λ begins when the phage binds to the surface of the cell and injects its linear DNA

► **Figure 26.5 The lytic cycle of phage T4, a virulent phage.** Phage T4 has almost 300 genes, which are transcribed and translated using the host cell's machinery. One of the first phage genes translated after the viral DNA enters the host cell codes for an enzyme that degrades the host cell's DNA (step 2); the phage DNA is protected from breakdown because it contains a modified form of cytosine that is not recognized by the phage enzyme. The entire lytic cycle, from the phage's first contact with the cell surface to cell lysis, takes only 20–30 minutes at 37°C.



genome (**Figure 26.6**). Within the host, the λ DNA molecule forms a circle. What happens next depends on the replicative mode: lytic cycle or lysogenic cycle. During a lytic cycle, the viral genes immediately turn the host cell into a λ -producing factory, and the cell soon lyses and releases its virus progeny. During a lysogenic cycle, however, the λ DNA molecule is incorporated into a specific site on the *E. coli* chromosome by viral proteins that break both circular DNA molecules and join them to each other. When integrated into the bacterial chromosome in this way, the viral DNA is known as a **prophage**. One prophage gene codes for a protein that prevents transcription of most of the other prophage genes. Thus, the phage genome is mostly silent within the bacterium. Every time the *E. coli* cell prepares to divide, it replicates the phage DNA along with its own chromosome such that each daughter cell inherits a prophage. A single infected cell can quickly give rise to a large population of bacteria carrying the virus in prophage form. This mechanism enables viruses to propagate without killing the host cells on which they depend.

The term *lysogenic* signifies that prophages are capable of generating active phages that lyse their host cells. This occurs when the λ genome (or that of another temperate phage) is induced to exit the bacterial chromosome and initiate a lytic cycle. An environmental signal, such as a certain chemical or

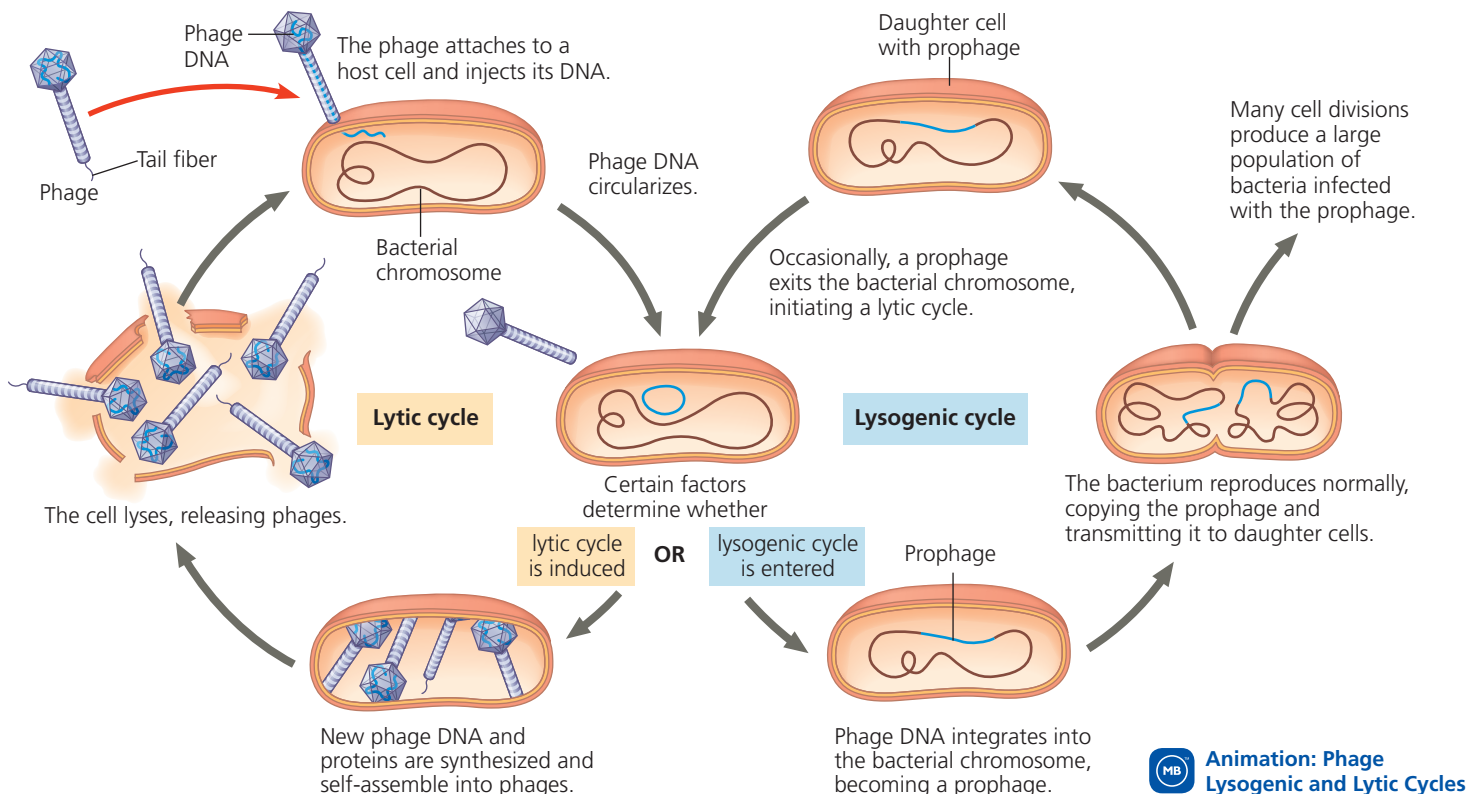
high-energy radiation, usually triggers the switchover from the lysogenic to the lytic mode.

In addition to the gene for the viral protein that prevents transcription, a few other prophage genes may be expressed during lysogeny. Expression of these genes may alter the host's phenotype, a phenomenon that can have important medical significance. For example, the three species of bacteria that cause the human diseases diphtheria, botulism, and scarlet fever would not be so harmful to humans without certain prophage genes that cause the host bacteria to make toxins. And the difference between the *E. coli* strain in our intestines and the O157:H7 strain that has caused several deaths by food poisoning appears to be the presence of toxin genes of prophages in the O157:H7 strain.

Bacterial Defenses Against Phages

After reading about the lytic cycle, you may have wondered why phages haven't exterminated all bacteria. Lysogeny is one major reason why bacteria have been spared from extinction caused by phages. Bacteria also have their own defenses against phages. First, natural selection favors bacterial mutants with surface proteins that are no longer recognized as receptors by a particular type of phage. Second, when phage DNA does enter a bacterium, the DNA

▼ **Figure 26.6 The lytic and lysogenic cycles of phage λ , a temperate phage.** After entering the bacterial cell and circularizing, the λ DNA can immediately initiate the production of a large number of progeny phages (lytic cycle) or integrate into the bacterial chromosome (lysogenic cycle). In most cases, phage λ follows the lytic pathway, which is similar to that detailed in Figure 26.5. However, once a lysogenic cycle begins, the prophage may be carried in the host cell's chromosome for many generations. Phage λ has one main tail fiber, which is short.



often is identified as foreign and cut up by cellular enzymes called **restriction enzymes**, which are so named because they *restrict* a phage's ability to replicate within the bacterium. (Restriction enzymes are used in molecular biology and DNA cloning techniques; see Concept 19.1.) The bacterium's own DNA is methylated in a way that prevents attack by its own restriction enzymes. A third defense is a system present in both bacteria and archaea called the *CRISPR-Cas system*.

The CRISPR-Cas system was discovered during a study of repetitive DNA sequences present in the genomes of many prokaryotes. These sequences, which puzzled scientists, were named clustered regularly interspaced short palindromic repeats (CRISPRs) because each sequence read the same forward and backward (a palindrome), with different stretches of "spacer DNA" in between the repeats. At first, scientists assumed the spacer DNA sequences were random and meaningless, but analysis by several research groups showed that each spacer sequence corresponded to DNA from a particular phage that had infected the cell. Further studies revealed that particular nuclease proteins interact with the CRISPR region. These nucleases, called Cas (CRISPR-associated) proteins, can identify and cut phage DNA, thereby defending the bacterium against phage infection.

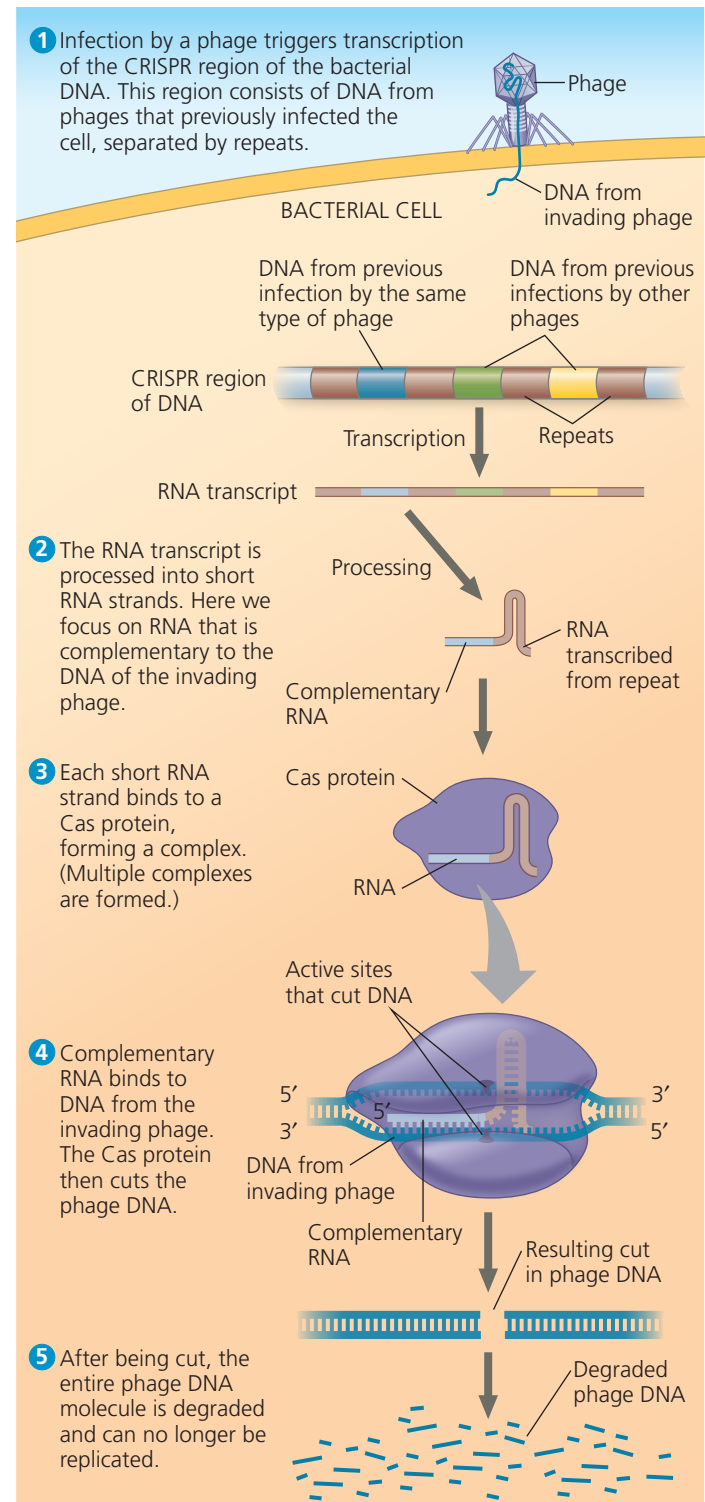
When a phage infects a bacterial cell that has the CRISPR-Cas system, the DNA of the invading phage is integrated into the genome between two repeat sequences. If the cell survives the infection, any further attempt by the same type of phage to infect this cell (or its offspring) triggers transcription of the CRISPR region into RNA molecules (**Figure 26.7**). These RNAs are cut into pieces and then bound by Cas proteins. The Cas protein uses a portion of the phage-related RNA as a homing device to identify the invading phage DNA and cut it, leading to its destruction. In Concept 19.1, you'll learn how this system is used in the laboratory to alter genes in other cells.

Just as natural selection favors bacteria that have receptors altered by mutation or that have enzymes that cut phage DNA, it also favors phage mutants that can bind to altered receptors or that are resistant to enzymes. Thus, the bacterium-phage relationship is in constant evolutionary flux.

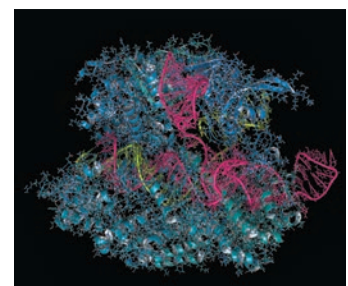
Replicative Cycles of Animal Viruses

Everyone has suffered from viral infections, whether cold sores, influenza, or the common cold. Like all viruses, those that cause illness in humans and other animals can replicate only inside host cells. Many variations on the basic scheme of viral infection and replication are represented among the animal viruses. One key variable is the nature of the viral genome (double- or single-stranded DNA or RNA). Another variable is the presence or absence of a membranous envelope. Whereas few bacteriophages have an envelope or RNA genome, many animal viruses have both. In fact, nearly all

▼ **Figure 26.7** The CRISPR-Cas system: a type of bacterial immune system.



► Computer model of CRISPR-Cas9 gene editing complex from *Streptococcus pyogenes*



animal viruses with RNA genomes have an envelope, as do some with DNA genomes. Rather than consider all the mechanisms of viral infection and replication, we will focus first on the roles of viral envelopes and then on the functioning of RNA as the genetic material of many animal viruses.

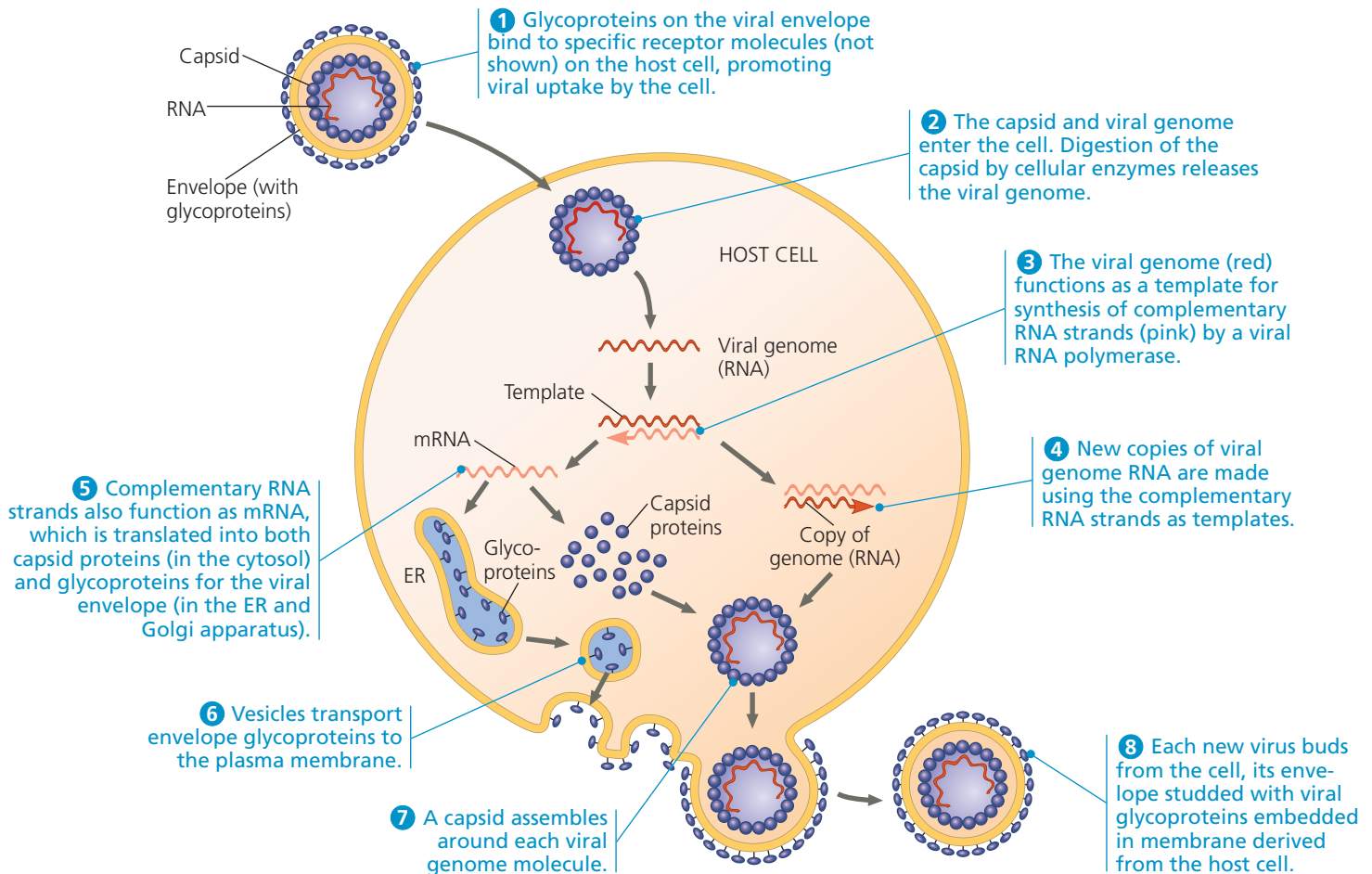
Viral Envelopes

An animal virus equipped with an envelope—that is, a membranous outer layer—uses it to enter the host cell. Protruding from the outer surface of this envelope are viral glycoproteins that bind to specific receptor molecules on the surface of a host cell. **Figure 26.8** outlines the events in the replicative cycle of an enveloped virus with an RNA genome. Ribosomes bound to the endoplasmic reticulum (ER) of the host cell make the protein parts of the envelope glycoproteins; cellular enzymes in the ER and Golgi apparatus then add the sugars. The resulting viral glycoproteins, embedded in membrane derived from the host cell, are transported to the cell surface. In a process much like exocytosis, new viral capsids are wrapped in membrane as they bud from the cell. In other words, the viral envelope is usually derived from

the host cell's plasma membrane, although all or most of the molecules of this membrane are specified by viral genes. The enveloped viruses are now free to infect other cells. This replicative cycle does not necessarily kill the host cell, in contrast to the lytic cycles of phages.

Some viruses have envelopes that are not derived from plasma membrane. Herpesviruses, for example, are temporarily cloaked in membrane derived from the nuclear envelope of the host; they then shed this membrane in the cytoplasm and acquire a new envelope made from membrane of the Golgi apparatus. These viruses have a double-stranded DNA genome and replicate within the host cell nucleus, using a combination of viral and cellular enzymes to replicate and transcribe their DNA. In the case of herpesviruses, copies of the viral DNA can remain behind as mini-chromosomes in the nuclei of certain nerve cells. There they remain latent until some sort of physical or emotional stress triggers a new round of active virus production. The infection of other cells by these new viruses causes the blisters characteristic of herpes, such as cold sores or genital sores. Once someone acquires a herpesvirus infection, flare-ups may recur throughout the person's life.

▼ **Figure 26.8 The replicative cycle of an enveloped RNA virus.** Shown here is a virus with a single-stranded RNA genome that functions as a template for synthesis of mRNA. Some enveloped viruses enter the host cell by fusion of the envelope with the cell's plasma membrane; others enter by endocytosis. For all enveloped RNA viruses, formation of new envelopes for progeny viruses occurs by the mechanism depicted in this figure.



Viral Genetic Material

Table 26.1 shows the common classification system for animal viruses, which is based on their genetic material: double- or single-stranded DNA, or double- or single-stranded RNA. Although some phages and most plant viruses are RNA viruses, the broadest variety of RNA genomes is found among the viruses that infect animals. There are three types of single-stranded RNA genomes found in animal viruses (classes IV–VI in Table 26.1). The genome of class IV viruses can directly

Class/Family	Envelope?	Examples That Cause Human Diseases
I. Double-Stranded DNA (dsDNA)		
Adenovirus (see Figure 26.3b)	No	Respiratory viruses
Papillomavirus	No	Warts, cervical cancer
Polyomavirus	No	Tumors
Herpesvirus	Yes	Herpes simplex I and II (cold sores, genital sores); varicella zoster (shingles, chicken pox); Epstein-Barr virus (mononucleosis, Burkitt's lymphoma)
Poxvirus	Yes	Smallpox virus; cowpox virus
II. Single-Stranded DNA (ssDNA)		
Parvovirus	No	B19 parvovirus (mild rash)
III. Double-Stranded RNA (dsRNA)		
Reovirus	No	Rotavirus (diarrhea); Colorado tick fever virus
IV. Single-Stranded RNA (ssRNA); Serves as mRNA		
Picornavirus	No	Rhinovirus (common cold); poliovirus; hepatitis A virus; other intestinal viruses
Coronavirus	Yes	Severe acute respiratory syndrome (SARS); Middle East respiratory syndrome (MERS)
Flavivirus	Yes	Zika virus (see Figure 26.10c); yellow fever virus; dengue virus; West Nile virus; hepatitis C virus
Togavirus	Yes	Chikungunya virus (see Figure 26.10b); rubella virus; equine encephalitis viruses
V. ssRNA; Serves as Template for mRNA Synthesis		
Filovirus	Yes	Ebola virus (hemorrhagic fever; see Figure 26.10a)
Orthomyxovirus	Yes	Influenza virus (see Figure 26.3c)
Paramyxovirus	Yes	Measles virus; mumps virus
Rhabdovirus	Yes	Rabies virus
VI. ssRNA; Serves as Template for DNA Synthesis		
Retrovirus	Yes	Human immunodeficiency virus (HIV/AIDS; see Figure 26.9); RNA tumor viruses (leukemia)

serve as mRNA and thus can be translated into viral protein immediately after infection. Figure 26.8 shows a virus of class V, in which the RNA genome serves instead as a *template* for mRNA synthesis. The RNA genome is transcribed into complementary RNA strands, which function both as mRNA and as templates for the synthesis of additional copies of genomic RNA. All viruses that use an RNA genome as a template for mRNA transcription require RNA → RNA synthesis. These viruses use a viral enzyme capable of carrying out this process; there are no such enzymes in most cells. The enzyme used in this process is encoded by the viral genome, and after its synthesis the protein is packaged during viral self-assembly with the genome inside the viral capsid.

The RNA animal viruses with the most complicated replicative cycles are the **retroviruses** (class VI). These viruses have an enzyme called **reverse transcriptase** that transcribes an RNA template into DNA, an RNA → DNA information flow that is the opposite of the usual direction. This unusual phenomenon is the source of the name retroviruses (*retro* means “backward”). Of particular medical importance is **HIV (human immunodeficiency virus)**, the retrovirus shown in Figure 26.1 that causes **AIDS (acquired immunodeficiency syndrome)**. HIV and other retroviruses are enveloped viruses that contain two identical molecules of single-stranded RNA and two molecules of reverse transcriptase.

The HIV replicative cycle (traced in **Figure 26.9**) is typical of a retrovirus. After HIV enters a host cell, its reverse transcriptase molecules are released into the cytoplasm, where they catalyze synthesis of viral DNA. The newly made viral DNA then enters the cell's nucleus and integrates into the DNA of a chromosome. The integrated viral DNA, called a **provirus**, never leaves the host's genome, remaining a permanent resident of the cell. (Recall that a prophage, in contrast, leaves the host's genome at the start of a lytic cycle.) The RNA polymerase of the host transcribes the proviral DNA into RNA molecules, which can function both as mRNA for the synthesis of viral proteins and as genomes for the new viruses that will be assembled and released from the cell. In Concept 47.4, we describe how HIV causes the deterioration of the immune system that occurs in AIDS.

Evolution of Viruses

EVOLUTION We began this chapter by asking whether or not viruses are alive. Viruses do not really fit our definition of living organisms. An isolated virus is biologically inert, unable to replicate its genes or regenerate its own ATP. Yet it has a genetic program written in the universal language of life. Do we think of viruses as nature's most complex associations of molecules or as the simplest forms of life? Either way, we must bend our usual definitions. Although viruses cannot replicate or carry out metabolic activities independently, their use of the genetic code makes it hard to deny their evolutionary connection to the living world.

1 The envelope glycoproteins enable the virus to bind to specific receptors (not shown) on certain white blood cells.

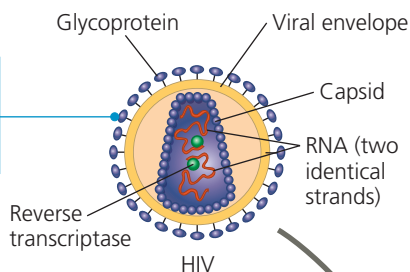
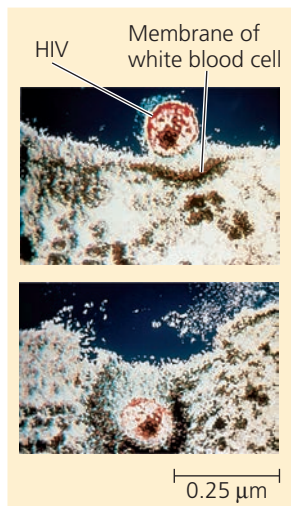
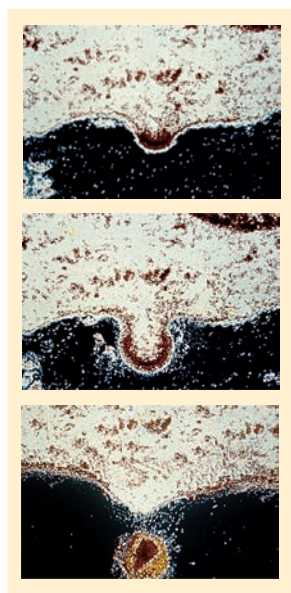


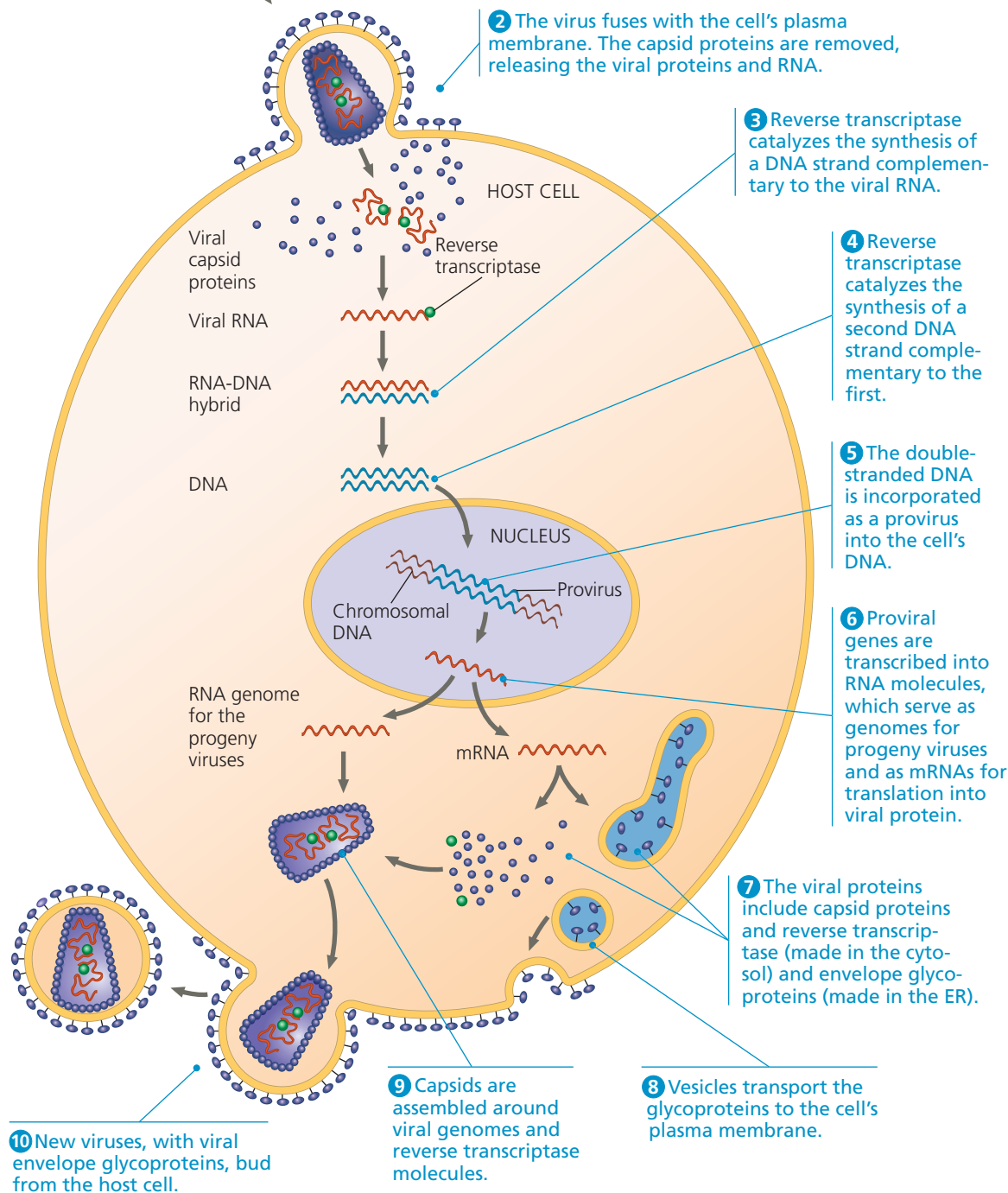
Figure 26.9 The replicative cycle of HIV, the retrovirus that causes AIDS. Note in step 5 that DNA synthesized from the viral RNA genome is integrated as a provirus into the host cell chromosomal DNA, a characteristic unique to retroviruses. For simplicity, the cell-surface proteins that act as receptors for HIV are not shown. The photos on the left (artificially colored TEMs) show HIV entering and leaving a human white blood cell.



HIV entering a cell



New HIV leaving a cell



MAKE CONNECTIONS ▶ Describe what is known about binding of HIV to immune system cells. (See Figure 8.8.) How was this discovered?



Animation: Retrovirus (HIV) Replicative Cycle

How did viruses originate? Viruses have been found that infect every form of life—not just bacteria, animals, and plants, but also archaea, fungi, and algae and other protists. Because they depend on cells for their own propagation, it seems likely that viruses are not the descendants of precellular forms of life but evolved—possibly multiple times—*after* the first cells appeared. Most molecular biologists favor the hypothesis that viruses originated from naked bits of cellular nucleic acids that moved from one cell to another, perhaps via injured cell surfaces. The evolution of genes coding for capsid proteins may have allowed viruses to bind cell membranes, thus facilitating the infection of uninjured cells.

Candidates for the original sources of viral genomes include plasmids and transposons. *Plasmids* are small, circular DNA molecules found in bacteria and in the unicellular eukaryotes called yeasts. Plasmids exist apart from and can replicate independently of the bacterial chromosome and are occasionally transferred between cells. *Transposons* are DNA segments that can move from one location to another within a cell's genome. Thus, plasmids, transposons, and viruses all share an important feature: They are *mobile genetic elements*. (We'll discuss plasmids in more detail in Concepts 19.1 and 27.2 and transposons in Concept 20.4.)

Consistent with this notion of pieces of DNA shuttling from cell to cell is the observation that a viral genome can have more in common with the genome of its host than with the genomes of viruses that infect other hosts. Indeed, some viral genes are essentially identical to genes of the host.

The debate about the origin of viruses was reinvigorated about 15 years ago by reports of one of the largest viruses yet discovered: Mimivirus is a double-stranded DNA (dsDNA) virus with an icosahedral capsid that is 400 nm in diameter, the size of a small bacterium. Its genome contains 1.2 million bases (Mb)—about 100 times as many as the influenza virus genome—and an estimated 1,000 genes. Perhaps the most surprising aspect of mimivirus, however, was that its genome included genes previously found only in cellular genomes. Some of these genes code for proteins involved in translation, DNA repair, protein folding, and polysaccharide synthesis. Whether mimivirus evolved *before* the first cells and then developed an exploitative relationship with them or evolved more recently and simply scavenged genes from its hosts is not yet settled. Since 2013 several even larger viruses have been discovered that cannot be classified with any existing known virus. One such virus is 1 μm (1,000 nm) in diameter, with a dsDNA genome of around 2–2.5 Mb, larger than that of some small eukaryotes. What's more, over 90% of its 2,000 or so genes are unrelated to cellular genes, inspiring the name it was given, pandoravirus. A second virus, called *Pithovirus sibericum*, with a diameter of 1.5 μm and 500 genes, was discovered in permanently frozen soil in Siberia. This virus, once thawed, was able to infect an amoeba after being frozen for 30,000 years! How these and all other viruses fit in the tree of life is an intriguing, unresolved question.

The ongoing evolutionary relationship between viruses and the genomes of their host cells is an association that continues to make viruses very useful experimental systems in molecular biology. Knowledge about viruses also allows many practical applications, since viruses have a tremendous impact on all organisms through their ability to cause disease.

CONCEPT CHECK 26.2

1. Compare the effect on the host cell of a lytic (virulent) phage and a lysogenic (temperate) phage.
2. **MAKE CONNECTIONS** > Compare the CRISPR-Cas system to the miRNA system discussed in Concept 18.3, including their mechanisms and their functions.
3. **MAKE CONNECTIONS** > The RNA virus in Figure 26.8 has a viral RNA polymerase that functions in step 3 of the virus's replicative cycle. Compare this with a cellular RNA polymerase in terms of template and overall function (see Figure 17.10).
4. Why is HIV called a retrovirus?
5. **VISUAL SKILLS** > Looking at Figure 26.9, imagine you are a researcher trying to combat HIV infection. What molecular processes could you attempt to block?

For suggested answers, see Appendix A.

CONCEPT 26.3

Viruses and prions are formidable pathogens in animals and plants

Diseases caused by viral infections afflict humans, agricultural crops, and livestock worldwide. Other smaller, less complex entities known as prions also cause disease in animals. We'll first discuss animal viruses.

Viral Diseases in Animals

A viral infection can produce symptoms by a number of different routes. Viruses may damage or kill cells by causing the release of hydrolytic enzymes from lysosomes. Some viruses cause infected cells to produce toxins that lead to disease symptoms, and some have molecular components that are toxic, such as envelope proteins. How much damage a virus causes depends partly on the ability of the infected tissue to regenerate by cell division. People usually recover completely from colds because the epithelium of the respiratory tract, which the viruses infect, can efficiently repair itself. In contrast, damage inflicted by poliovirus to mature nerve cells is permanent because these cells do not divide and usually cannot be replaced. Many of the temporary symptoms associated with viral infections, such as fever and body aches, actually result from the body's own efforts to defend itself against infection rather than from cell death caused by the virus.

The immune system is a critical part of the body's natural defenses (see Chapter 47). It is also the basis for the major medical tool used to prevent viral infections—vaccines. A **vaccine** is a harmless derivative of a pathogen that stimulates

How do such viruses burst on the human scene, giving rise to harmful diseases that were previously rare or even unknown? Three processes contribute to the emergence of viral diseases. The first, and perhaps most important, is the mutation of existing viruses. RNA viruses tend to have an unusually high rate of mutation because viral RNA polymerases do not proofread and correct errors in replicating their RNA genomes. Some mutations change existing viruses into new genetic varieties (strains) that can cause disease, even in individuals who are immune to the ancestral virus. For instance, seasonal flu epidemics are caused by new strains of influenza virus genetically different enough from earlier strains that people have little immunity to them. You'll see an example of this process in the **Scientific Skills Exercise**, where you'll analyze genetic changes in variants of the H1N1 flu virus and correlate them with spread of the disease.

A second process that can lead to the emergence of viral diseases is the dissemination of a viral disease from a small, isolated human population. For instance, AIDS went unnamed and virtually unnoticed for decades before it began to spread around the world. In this case, technological and social factors, including affordable international travel, blood transfusions, sexual promiscuity, and the abuse of intravenous drugs, allowed a previously rare human disease to become a global scourge.



Interview with David Satcher: The role of the CDC in recognizing AIDS and in public health

A third source of new viral diseases in humans is the spread of existing viruses from other animals. Scientists estimate that about three-quarters of new human diseases originate in this way. Animals that harbor and can transmit a particular virus but are generally unaffected by it are said to act as a natural reservoir for that virus. For example, the H1N1 virus that caused the 2009 flu pandemic mentioned earlier was likely passed to humans from pigs; for this reason, the disease it caused was originally called “swine flu.”

In general, flu epidemics provide an instructive example of the effects of viruses moving between species. There are three types of influenza virus: types B and C, which infect only humans and have never caused an epidemic, and type A, which infects a wide range of animals, including birds, pigs, horses, and humans. Influenza A strains have caused four major flu epidemics among humans in the last 100 years. The worst was the first one, the “Spanish flu” pandemic of 1918–1919, which killed 40–50 million people, including many World War I soldiers.

Different strains of influenza A are given standardized names; for example, both the strain that caused the 1918 flu and the one that caused the 2009 pandemic flu are called H1N1. The name identifies which forms of two viral surface proteins are present: hemagglutinin (HA) and neuraminidase (NA). There are 16 different types of hemagglutinin, a protein that helps the flu virus attach to host cells, and 9 types

of neuraminidase, an enzyme that helps release new virus particles from infected cells. Waterbirds have been found that carry viruses with all possible combinations of HA and NA. Variations of the hemagglutinin protein are used each year to generate vaccines against the strains predicted most likely to occur the next year.

A likely scenario for the 1918 pandemic and others is that the virus mutated as it passed from one host species to another. When an animal like a pig or a bird is infected with more than one strain of flu virus, the different strains can undergo genetic recombination if the RNA molecules making up their genomes mix and match during viral assembly. Pigs were probably the main hosts for recombination that led to the 2009 flu virus, which turns out to contain sequences from bird, pig, and human flu viruses. Coupled with mutation, these reassortments can lead to the emergence of a viral strain capable of infecting human cells. People who have never been exposed to that particular strain before will lack immunity, and the recombinant virus has the potential to be highly pathogenic. If such a flu virus recombines with viruses that circulate widely among humans, it may acquire the ability to spread easily from person to person, dramatically increasing the potential for a major human outbreak.

The many avian flu viruses carried by wild and domestic birds pose a potential long-term threat. A case in point is an H5N1 virus; the first transmission of H5N1 from birds to humans was documented in Hong Kong in 1997. Since then, the overall mortality rate due to H5N1 has been greater than 50% of those infected—an alarming number. Also, the host range of H5N1 is expanding, which provides increasing chances for reassortment between different strains. If the H5N1 avian flu virus evolves so that it can spread easily from person to person, it could represent a major global health threat akin to that of the 1918 pandemic.

How easily could this happen? In 2011, scientists working with ferrets, small mammals that are animal models for human flu, found out that only a few mutations of the avian flu virus would allow infection of cells in the human nasal cavity and windpipe. Furthermore, when the scientists transferred nasal swabs serially from ferret to ferret, the virus became transmissible through the air. Reports of this startling discovery at a scientific conference ignited a firestorm of debate about whether to publish the results and led to an ongoing reevaluation of the federal policies governing this type of experiment in the United States. The risks of doing this type of research (what if the new virus escapes or the procedure falls into the hands of bioterrorists?) must be considered in relation to the risks of not doing it—the possibility that we will be unable to combat new, more transmissible viruses because we lack an understanding of how they develop.

As we have seen, emerging viruses are generally not new; rather, they are existing viruses that mutate, disseminate more widely in the current host species, or spread to new host species. Changes in host behavior or environmental changes

SCIENTIFIC SKILLS EXERCISE

Analyzing a Sequence-Based Phylogenetic Tree to Understand Viral Evolution

How Can Sequence Data Be Used to Track Flu Virus Evolution?

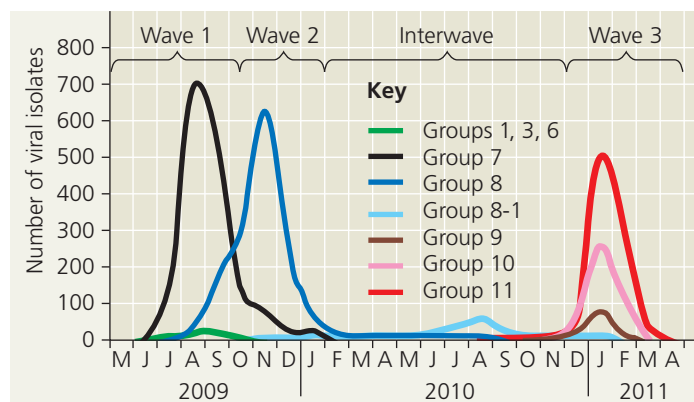
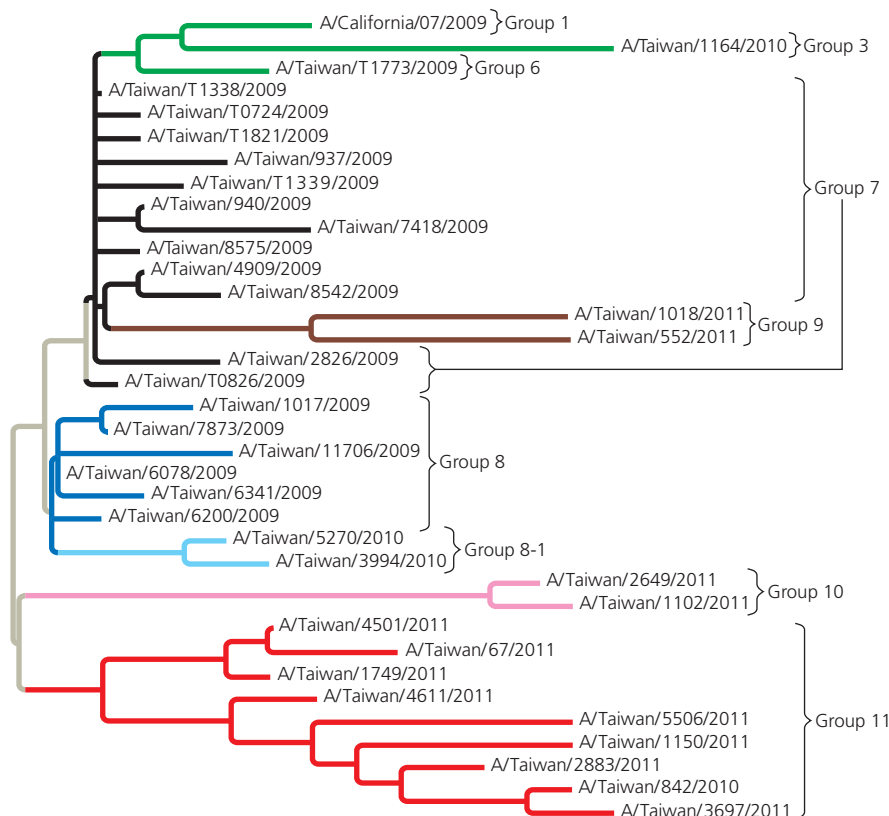
In 2009, an influenza A H1N1 virus caused a pandemic, and the virus has continued to resurface in outbreaks across the world. Researchers in Taiwan were curious about why the virus kept appearing despite widespread flu vaccine initiatives. They hypothesized that newly evolved variant strains of the H1N1 virus were able to evade human immune system defenses. To test this hypothesis, they needed to determine if each wave of flu infection was caused by a different H1N1 variant strain.

How the Experiment Was Done

Scientists obtained the genome sequences for 4,703 virus isolates collected from patients with H1N1 flu in Taiwan. They compared the sequences in different strains for the viral hemagglutinin (HA) gene, and based on mutations that had occurred, arranged the isolates into a phylogenetic tree (see Figure 22.5 for information on how to read phylogenetic trees).



▲ H1N1 flu vaccination.



▲ Scientists graphed the number of isolates by the month and year of isolate collection to show the period in which each viral variant was actively causing illness in people.

Data from the Experiment In the phylogenetic tree, each branch tip is one variant strain of the H1N1 virus with a unique HA gene sequence. The tree is a way to visualize a working hypothesis about the evolutionary relationships between H1N1 variants.

INTERPRET THE DATA

- The phylogenetic tree shows the hypothesized evolutionary relationship between the variant strains of H1N1 virus. The more closely connected two variants are, the more alike they are in terms of HA gene sequence. Each fork in a branch, called a node, shows where two lineages separate due to different accumulated mutations. The length of the branches is a measure of how many sequence differences there are between the variants, indicating how distantly related they are. Referring to the phylogenetic tree, which variants are more closely related to each other: A/Taiwan/1018/2011 and A/Taiwan/552/2011 or A/Taiwan/1018/2011 and A/Taiwan/8542/2009? Explain your answer.
- The scientists arranged the branches into groups made up of one ancestral variant and all of its descendant, mutated variants. They are color-coded in the figure. Using group 11 as an example, trace the lineage of its variants. (a) Do all of the nodes have the same number of branches or branch tips? (b) Are all of the branches in the group the same length? (c) What do these results indicate?
- The graph at the lower left shows the number of isolates collected (each from an ill patient) on the y-axis and the month and year that the isolates were collected on the x-axis. Each group of variants is plotted separately with a line color that matches the tree diagram. (a) Which group of variants was the earliest to cause the first wave of H1N1 flu in over 100 patients in Taiwan? (b) After a group of variants had a peak number of infections, did members of that same group cause another (later) wave of infection? (c) One variant in group 1 (green, uppermost branch) was used to make a vaccine that was distributed very early in the pandemic. Based on the graphed data, does it look like the vaccine was effective?
- Groups 9, 10, and 11 all had H1N1 variants that caused a large number of infections at the same time in Taiwan. Does this mean that the scientists' hypothesis, that new variants cause new waves of infection, was incorrect? Explain your answer.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Data from J.-R. Yang et al., New variants and age shift to high fatality groups contribute to severe successive waves in the 2009 influenza pandemic in Taiwan, *PLoS ONE* 6(11): e28288 (2011).

can increase the viral traffic responsible for emerging diseases. For instance, new roads built through remote areas can allow viruses to spread between previously isolated human populations. Also, the destruction of forests to expand cropland can bring humans into contact with other animals that may host viruses capable of infecting humans. Finally, genetic mutations and changes in host ranges can allow viruses to jump from one species to another. Many viruses, including chikungunya, mentioned earlier, can be transmitted by mosquitoes. A dramatic expansion of the disease caused by chikungunya occurred in the mid-2000s when a mutation in the virus allowed it to infect not only the mosquito species *Aedes aegypti*, but also the related *Aedes albopictus*. Promotion of the use of insecticides and mosquito netting over beds are crucial tools in public health attempts to prevent diseases carried by mosquitoes (**Figure 26.11**).

▼ **Figure 26.11 Mosquitoes as vectors for disease.**

Mosquitoes transmit viruses when they feed on infected blood from one person and then bite other people. Mosquito netting is an important means of preventing infection in affected areas.



Recently, scientists have become concerned about the possible effects of climate change on worldwide viral transmission. Dengue fever, also mosquito-borne, has appeared in Florida and Portugal, regions where it had not been seen before. The possibility that global climate change has allowed mosquito species carrying these viruses to expand their ranges and interact more is troubling because of the increased chance of a mutation allowing a virus species to jump to a new host. This is an area of active research by scientists applying climate change models to what is known about the habitat requirements of mosquito species.

Viral Diseases in Plants

More than 2,000 types of viral diseases of plants are known, and together they account for an estimated annual loss of \$15 billion worldwide due to their destruction of agricultural and horticultural crops. Common signs of viral infection

include bleached or brown spots on leaves and fruits (**Figure 26.12**), stunted growth, and damaged flowers or roots, all of which can diminish the yield and quality of crops.

► **Figure 26.12 Immature tomato infected by a virus.**



Plant viruses have the same basic structure and mode of replication as animal viruses. Most plant viruses discovered thus far, including tobacco mosaic virus (TMV), have an RNA genome. Many have a helical capsid, like TMV, while others have an icosahedral capsid (see Figure 26.3b).

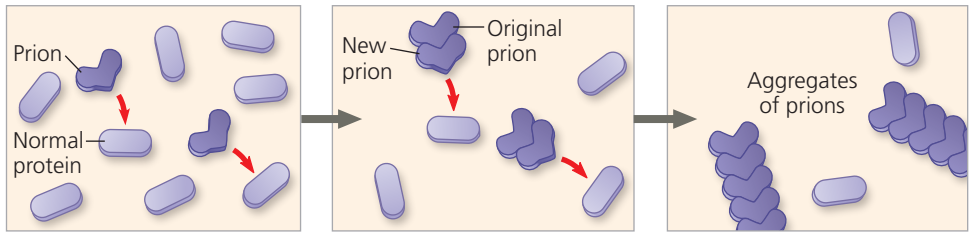
Viral diseases of plants spread by two major routes. In the first route, called *horizontal transmission*, a plant is infected from an external source of the virus. Because the invading virus must get past the plant's outer protective layer of cells (the epidermis), a plant becomes more susceptible to viral infections if it has been damaged by wind, injury, or herbivores. Herbivores, especially insects, pose a double threat because they can also act as carriers of viruses, transmitting disease from plant to plant. Moreover, farmers and gardeners may transmit plant viruses inadvertently on pruning shears and other tools. The other route of viral infection is *vertical transmission*, in which a plant inherits a viral infection from a parent. Vertical transmission can occur in asexual propagation (for example, through cuttings) or in sexual reproduction via infected seeds.

Once a virus enters a plant cell and begins replicating, viral genomes and associated proteins can spread throughout the plant by means of plasmodesmata, the cytoplasmic connections that penetrate the walls between adjacent plant cells (see Figure 36.19). The passage of viral macromolecules from cell to cell is facilitated by virally encoded proteins that cause enlargement of plasmodesmata. Scientists have not yet devised cures for most viral plant diseases. Consequently, research efforts are focused largely on reducing the transmission of such diseases and on breeding resistant varieties of crop plants.

Prions: Proteins as Infectious Agents

The viruses we have discussed in this chapter are infectious agents that spread diseases, and their genetic material is composed of nucleic acids, whose ability to be replicated is well known. Surprisingly, there are also *proteins* that are known to be infectious. Proteins called **prions** appear to cause a number of degenerative brain diseases in various animal species. These diseases include scrapie in sheep; mad cow disease, which has plagued the European beef industry in recent years; and Creutzfeldt-Jakob disease in humans, which has caused the death of some 175 people in the United Kingdom since 1996. Prions can be transmitted in food, as may occur when people eat prion-laden beef from cattle with mad cow disease. Kuru, another human disease caused by prions, was identified in the early 1900s among the South Fore natives of New Guinea. A kuru epidemic peaked there in the 1960s, puzzling scientists, who at first thought the disease had a genetic basis.

► **Figure 26.13 Model for how prions propagate.** Prions are misfolded versions of normal brain proteins. When a prion contacts a normally folded version of the same protein, it may induce the normal protein to assume the abnormal shape. The resulting chain reaction may continue until high levels of prion aggregation cause cellular malfunction and eventual degeneration of the brain.



Animation: Prions: Characteristics
Animation: Prions: Diseases

Eventually, however, anthropological investigations ferreted out how the disease was spread: ritual cannibalism, a widespread practice among South Fore natives at that time.

Two characteristics of prions are especially alarming. First, prions act very slowly, with an incubation period of at least ten years before symptoms develop. The lengthy incubation period prevents sources of infection from being identified until long after the first cases appear, allowing many more infections to occur. Second, prions are virtually indestructible; they are not destroyed or deactivated by heating to normal cooking temperatures. To date, there is no known cure for prion diseases, and the only hope for developing effective treatments lies in understanding the process of infection.

How can a protein, which cannot replicate itself, be a transmissible pathogen? According to the leading model, a prion is a misfolded form of a protein normally present in brain cells. When the prion gets into a cell containing the normal form of the protein, the prion somehow converts normal protein molecules to the misfolded prion versions. Several prions then aggregate into a complex that can convert other normal

proteins to prions, which join the chain (**Figure 26.13**). Prion aggregation interferes with normal cellular functions and causes disease symptoms. This model was greeted with much skepticism when it was first proposed by Stanley Prusiner in the early 1980s, but it is now widely accepted. Prusiner was awarded the Nobel Prize in 1997 for his work on prions. He has recently proposed that prions are also involved in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. There are many outstanding questions about these small infectious agents.

CONCEPT CHECK 26.3

1. Describe two ways in which a preexisting virus can become an emerging virus.
2. Prions do not contain genetic material and, therefore, cannot replicate themselves. How do they then cause diseases?
3. **WHAT IF? >** TMV has been isolated from virtually all commercial tobacco products. Why, then, is TMV infection not an additional hazard for smokers?

For suggested answers, see Appendix A.

26 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 26.1

A virus consists of a nucleic acid surrounded by a protein coat (pp. 609–611)

- Researchers discovered viruses in the late 1800s by studying a plant disease, tobacco mosaic disease.
- A **virus** is a small nucleic acid genome enclosed in a protein **capsid** and sometimes a membranous **viral envelope**. The genome may be single- or double-stranded DNA or RNA.

? Are viruses generally considered living or nonliving? Explain.



VOCAB
SELF-QUIZ
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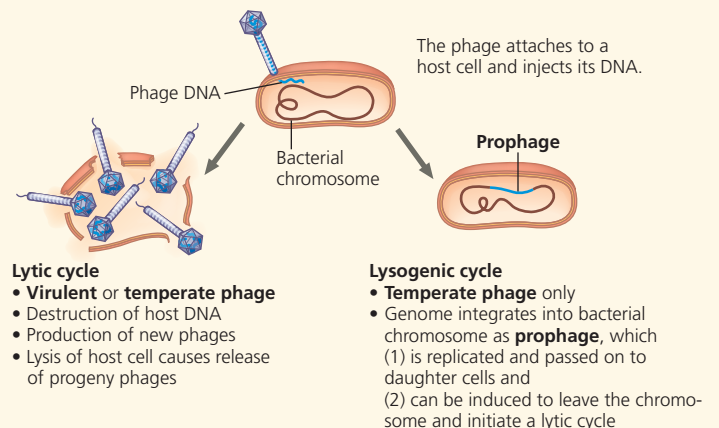
CONCEPT 26.2

Viruses replicate only in host cells (pp. 611–618)

- Viruses use enzymes, ribosomes, and small molecules of host cells to synthesize progeny viruses during replication.

Go to MasteringBiology™ for Videos, Animations, Vocab Self-Quiz, Practice Tests, and more in the Study Area.

- Each type of virus has a characteristic **host range**, affected by whether cell-surface proteins are present that viral surface proteins can bind to.
- **Phages** (viruses that infect bacteria) can replicate by two alternative mechanisms: the **lytic cycle** and the **lysogenic cycle**.



- Bacteria have various ways of defending themselves against phage infections, including the CRISPR-Cas system.
- Many animal viruses have an envelope. **Retroviruses** (such as **HIV**) use the enzyme **reverse transcriptase** to copy their RNA genome into DNA, which can be integrated into the host genome as a **provirus**.
- Since viruses can replicate only within cells, they probably evolved after the first cells appeared, perhaps as packaged fragments of cellular nucleic acid.

? Describe enzymes that are not found in most cells but are necessary for the replication of certain types of viruses.

CONCEPT 26.3

Viruses and prions are formidable pathogens in animals and plants (pp. 618–623)

- Symptoms of viral diseases may be caused by direct viral harm to cells or by the body's immune response. **Vaccines** stimulate the immune system to defend the host against specific viruses.
- An **epidemic**, a widespread outbreak of a disease, can become a **pandemic**, a global epidemic.
- Outbreaks of emerging viral diseases in humans are usually not new, but rather are caused by existing viruses that expand their host territory. The H1N1 2009 flu virus was a new combination of pig, human, and avian viral genes that caused a pandemic. The H5N1 avian flu virus has the potential to cause a high-mortality flu pandemic.
- Viruses enter plant cells through damaged cell walls (horizontal transmission) or are inherited from a parent (vertical transmission).
- **Prions** are slow-acting, virtually indestructible infectious proteins that cause brain diseases in mammals.

? What aspect of an RNA virus makes it more likely than a DNA virus to become an emerging virus?

TEST YOUR UNDERSTANDING



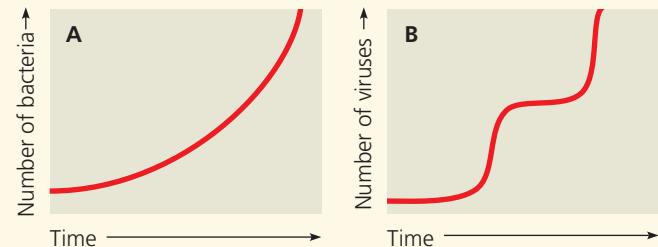
Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

6. **DRAW IT** Redraw Figure 26.8 to show the replicative cycle of a virus with a single-stranded genome that can function as mRNA (a class IV virus).



PRACTICE TEST
goo.gl/iAsVgL

7. **EVOLUTION CONNECTION** The success of some viruses lies in their ability to evolve rapidly within the host. Such viruses evade the host's defenses by mutating and producing many altered progeny viruses before the body can mount an attack. Thus, the viruses present late in infection differ from those that initially infected the body. Discuss this as an example of evolution in microcosm. Which viral lineages tend to predominate?
8. **SCIENTIFIC INQUIRY** When bacteria infect an animal, the number of bacteria in the body increases in an exponential fashion (graph A). After infection by a virulent animal virus with a lytic replicative cycle, there is no evidence of infection for a while. Then the number of viruses rises suddenly and subsequently increases in a series of steps (graph B). Explain the difference in the curves.



9. **WRITE ABOUT A THEME: ORGANIZATION** While viruses are considered by most scientists to be nonliving, they do show some characteristics of life, including the correlation of structure and function. In a short essay (100–150 words), discuss how the structure of a virus correlates with its function.
10. **SYNTHESIZE YOUR KNOWLEDGE**



Oseltamivir (Tamiflu), an antiviral drug prescribed for influenza, inhibits the enzyme neuraminidase. Explain how this drug could prevent infection in someone exposed to the flu or could shorten the course of flu in an infected patient (the reasons for which it is prescribed).

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Prokaryotes

27

▲ **Figure 27.1** Why is this lake's water pink?

KEY CONCEPTS

- 27.1** Structural and functional adaptations contribute to prokaryotic success
- 27.2** Rapid reproduction, mutation, and genetic recombination promote genetic diversity in prokaryotes
- 27.3** Diverse nutritional and metabolic adaptations have evolved in prokaryotes
- 27.4** Prokaryotes have radiated into a diverse set of lineages
- 27.5** Prokaryotes play crucial roles in the biosphere
- 27.6** Prokaryotes have both beneficial and harmful impacts on humans



◀ Archaea in the genus *Halobacterium*

Masters of Adaptation

At certain times of year, the Laguna Salada de Torrevieja in Spain (the “Salty Lagoon”) appears pink (**Figure 27.1**), a sign that its waters are many times saltier than seawater. Yet despite these harsh conditions, the dramatic color is caused not by minerals or other nonliving sources, but by living things. What organisms can live in such an inhospitable environment, and how do they do it?

The pink color in the Laguna Salada de Torrevieja comes from trillions of prokaryotes in the domains Archaea and Bacteria, including archaea in the genus *Halobacterium*. These archaea have red membrane pigments, some of which capture light energy that is used to drive ATP synthesis. *Halobacterium* species are among the most salt-tolerant organisms on Earth; they thrive in salinities that dehydrate and kill other cells. A *Halobacterium* cell compensates for water lost through osmosis by pumping potassium ions (K^+) into the cell until the ionic concentration inside the cell matches the concentration outside.

Like *Halobacterium*, many other prokaryotes can tolerate extreme conditions. Examples include *Deinococcus radiodurans*, which can survive 3 million rads of radiation (3,000 times the dose fatal to humans), and *Picrophilus oshimae*, which can grow at a pH of 0.03 (acidic enough to dissolve metal). Other prokaryotes live in environments that are too cold or too hot for most other organisms, and some have even been found living in rocks 3.2 km (2 miles) below Earth’s surface.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.



Get Ready for This Chapter

Prokaryotic species are also very well adapted to more “normal” habitats—the lands and waters in which most other species are found. Their ability to adapt to a broad range of habitats helps explain why prokaryotes are the most abundant organisms on Earth. Indeed, the number of prokaryotes in a handful of fertile soil is greater than the number of people who have ever lived. In this chapter, we’ll examine the adaptations, diversity, and enormous ecological impact of these remarkable organisms.

CONCEPT 27.1

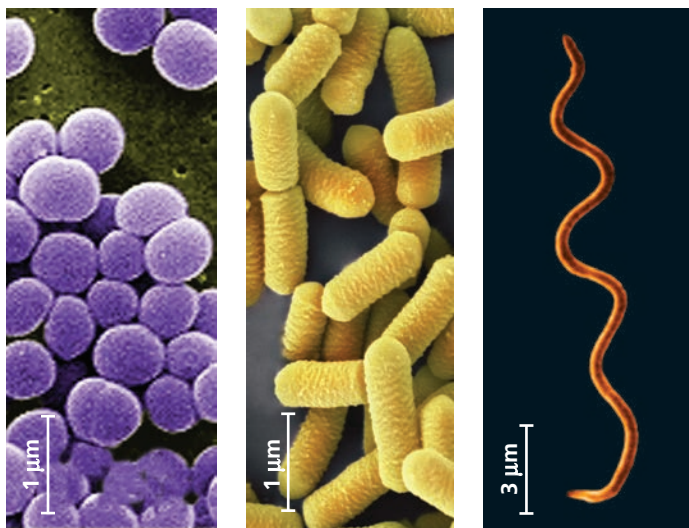
Structural and functional adaptations contribute to prokaryotic success

The first organisms to inhabit Earth were prokaryotes that lived 3.5 billion years ago (see Concept 25.3). Throughout their long evolutionary history, prokaryotic populations have been (and continue to be) subjected to natural selection in all kinds of environments, resulting in their enormous diversity today.

We’ll begin by describing prokaryotes. Most prokaryotes are unicellular, although the cells of some species remain attached to each other after cell division. Prokaryotic cells typically have diameters of 0.5–5 μm , much smaller than the 10- to 100- μm diameter of many eukaryotic cells. (One notable exception, *Thiomargarita namibiensis*, can be as large as 750 μm in diameter—bigger than a poppy seed.) Prokaryotic cells have a variety of shapes (Figure 27.2).

✓ Figure 27.2 The most common shapes of prokaryotes.

(a) Cocci (singular, *coccus*) are spherical prokaryotes. They occur singly, in pairs (diplococci), in chains of many cells (streptococci), and in clusters resembling bunches of grapes (staphylococci). (b) Bacilli (singular, *bacillus*) are rod-shaped prokaryotes. They are usually solitary, but in some forms the rods are arranged in chains (streptobacilli). (c) Spiral prokaryotes include spirilla, which range from comma-like shapes to loose coils, and spirochetes (shown here), which are corkscrew-shaped (colorized SEMs).



(a) Spherical

(b) Rod-shaped

(c) Spiral

Finally, although they are unicellular and small, prokaryotes are well organized, achieving all of an organism’s life functions within a single cell.

Cell-Surface Structures

A key feature of nearly all prokaryotic cells is the cell wall, which maintains cell shape, protects the cell, and prevents it from bursting in a hypotonic environment (see Figure 8.12). In a hypertonic environment, most prokaryotes lose water and shrink away from their wall (plasmolyze). Such water losses can inhibit cell reproduction. Thus, salt can be used to preserve foods because it causes food-spoiling prokaryotes to lose water, preventing them from rapidly multiplying.

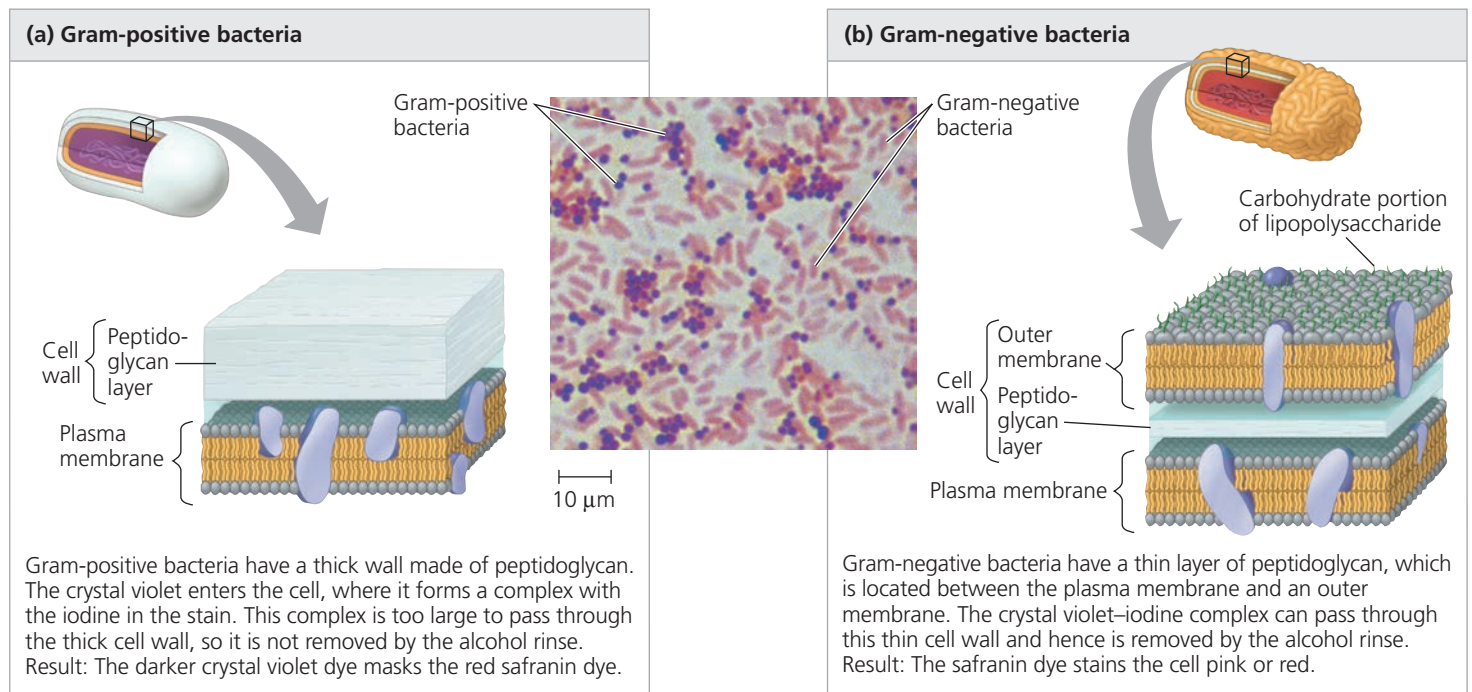
The cell walls of prokaryotes differ in structure from those of eukaryotes. In eukaryotes that have cell walls, such as plants and fungi, the walls are usually made of cellulose or chitin (see Concept 5.2). In contrast, most bacterial cell walls contain **peptidoglycan**, a polymer composed of modified sugars cross-linked by short polypeptides. This molecular fabric encloses the entire bacterium and anchors other molecules that extend from its surface. Archaeal cell walls contain a variety of polysaccharides and proteins but lack peptidoglycan.

Using a technique called the **Gram stain**, developed by the 19th-century Danish physician Hans Christian Gram, scientists can categorize many bacterial species according to differences in cell wall composition. To do this, samples are first stained with crystal violet dye and iodine, then rinsed in alcohol, and finally stained with a red dye such as safranin that enters the cell and binds to its DNA. The structure of a bacterium’s cell wall determines the staining response (Figure 27.3). **Gram-positive** bacteria have relatively simple walls composed of a thick layer of peptidoglycan. The walls of **gram-negative** bacteria have less peptidoglycan and are structurally more complex, with an outer membrane that contains lipopolysaccharides (carbohydrates bonded to lipids).

Gram staining is a valuable tool in medicine for quickly determining if a patient’s infection is due to gram-negative or to gram-positive bacteria. This information has treatment implications. The lipid portions of the lipopolysaccharides in the walls of many gram-negative bacteria are toxic, causing fever or shock. Furthermore, the outer membrane of a gram-negative bacterium helps protect it from the body’s defenses. Gram-negative bacteria also tend to be more resistant than gram-positive species to antibiotics because the outer membrane impedes entry of the drugs. However, certain gram-positive species have virulent strains that are resistant to one or more antibiotics. (Figure 21.14 discusses one example: methicillin-resistant *Staphylococcus aureus*, or MRSA, which can cause lethal skin infections.)

The effectiveness of certain antibiotics, such as penicillin, derives from their inhibition of peptidoglycan cross-linking. The resulting cell wall may not be functional, particularly in

✓ **Figure 27.3 Gram staining.**



gram-positive bacteria. Such drugs destroy many species of pathogenic bacteria without adversely affecting human cells, which do not have peptidoglycan.

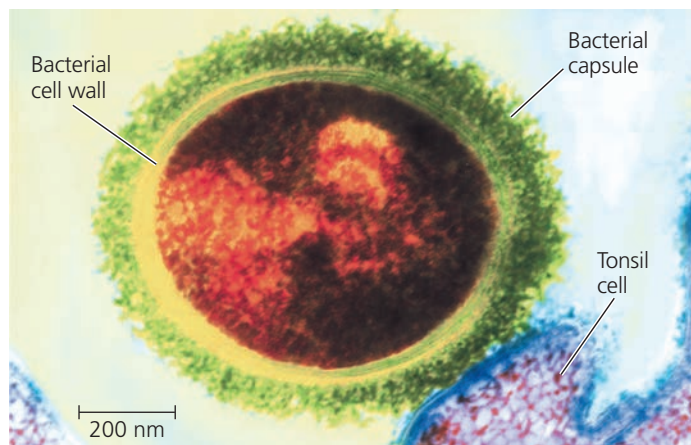
The cell wall of many prokaryotes is surrounded by a sticky layer of polysaccharide or protein. This layer is called a **capsule** if it is dense and well-defined (**Figure 27.4**) or a *slime layer* if it is not as well organized. Both kinds of sticky outer layers enable prokaryotes to adhere to their substrate or to other individuals in a colony. Some capsules and slime layers protect against dehydration, and some shield pathogenic prokaryotes from attacks by their host's immune system.

In another way of withstanding harsh conditions, certain bacteria develop resistant cells called **endospores** when they

lack water or essential nutrients (**Figure 27.5**). The original cell produces a copy of its chromosome and surrounds that copy with a multilayered structure, forming the endospore. Water is removed from the endospore, and its metabolism halts. The original cell then lyses, releasing the endospore. Most endospores are so durable that they can survive in boiling water; killing them requires heating lab equipment to 121°C under high pressure. In less hostile environments, endospores can remain dormant but viable for centuries, able to rehydrate and resume metabolism when their environment improves.

Finally, some prokaryotes stick to their substrate or to one another by means of hairlike appendages called **fimbriae**

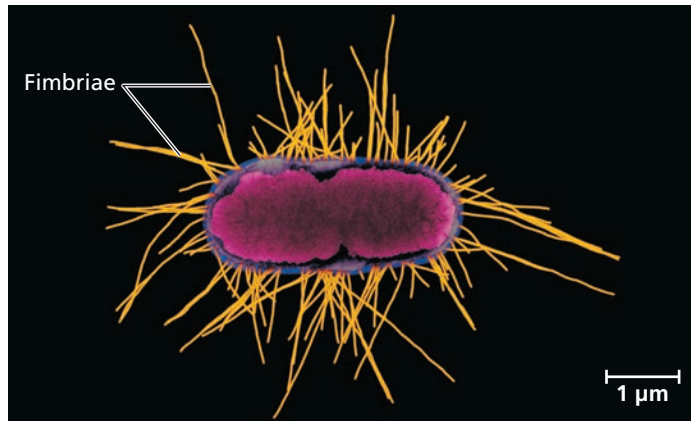
✓ **Figure 27.4 Capsule.** The polysaccharide capsule around this *Streptococcus* bacterium enables the prokaryote to attach to cells in the respiratory tract—in this colorized TEM, a tonsil cell.



✓ **Figure 27.5 An endospore.** *Bacillus anthracis*, the bacterium that causes the disease anthrax, produces endospores (TEM). An endospore's protective, multilayered coat helps it survive in the soil for years.



▼ **Figure 27.6 Fimbriae.** These numerous protein-containing appendages enable some prokaryotes to attach to surfaces or to other cells (colorized TEM).



(singular, *fimbria*) (**Figure 27.6**). For example, the bacterium that causes gonorrhea, *Neisseria gonorrhoeae*, uses fimbriae to fasten itself to the mucous membranes of its host. Fimbriae are usually shorter and more numerous than **pili** (singular, *pilus*), appendages that pull two cells together prior to DNA transfer from one cell to the other (see Figure 27.12); pili are sometimes referred to as *sex pili*.

Motility

About half of all prokaryotes are capable of **taxis**, a directed movement toward or away from a stimulus (from the Greek *taxis*, to arrange). For example, prokaryotes that exhibit *chemotaxis* change their movement pattern in response to chemicals. They may move *toward* nutrients or oxygen (positive chemotaxis) or *away from* a toxic substance (negative chemotaxis). Some species can move at velocities exceeding 50 μm/sec—up to 50 times their body length per second. For perspective, consider that a person 1.7 m tall moving that fast would be running 306 km (190 miles) per hour!

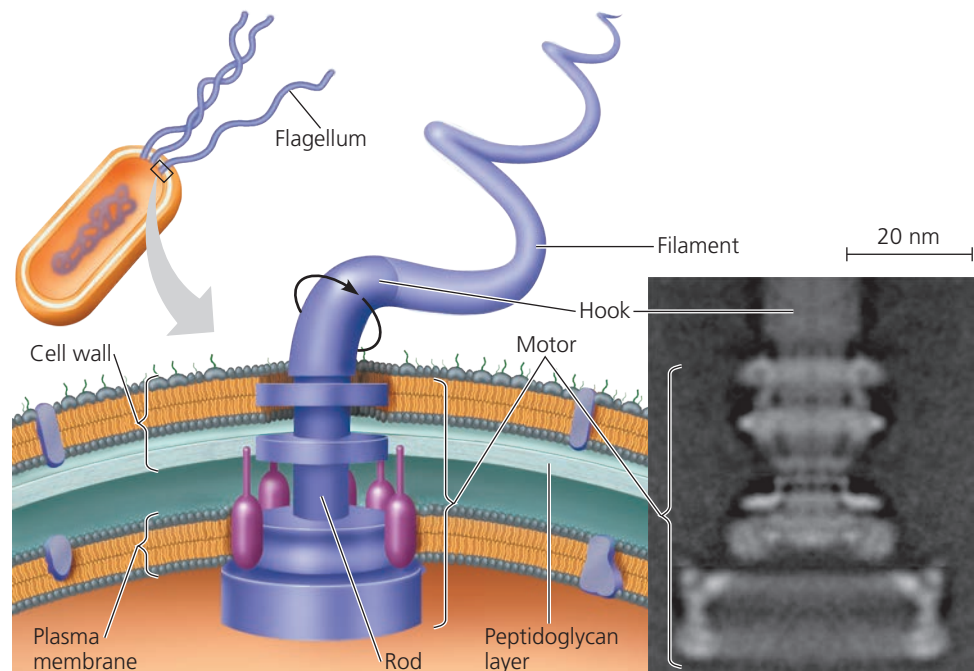
Of the various structures that enable prokaryotes to move, the most common are flagella (**Figure 27.7**). Flagella (singular, *flagellum*) may be scattered over the entire surface of the cell or concentrated at one or both ends. Prokaryotic flagella differ greatly from eukaryotic flagella: They are one-tenth the width and typically are not covered by an extension of the plasma membrane (see Figure 7.24). The flagella of prokaryotes and eukaryotes

also differ in their molecular composition and their mechanism of propulsion. Among prokaryotes, bacterial and archaeal flagella are similar in size and rotational mechanism, but they are composed of entirely different and unrelated proteins. Overall, these structural and molecular comparisons indicate that the flagella of bacteria, archaea, and eukaryotes arose independently. Since current evidence shows that the flagella of organisms in the three domains perform similar functions but are not related by common descent, they are described as analogous, not homologous, structures (see Concept 21.2).

Evolutionary Origins of Bacterial Flagella

The bacterial flagellum shown in Figure 27.7 has three main parts (the motor, hook, and filament) that are themselves composed of 42 different kinds of proteins. How could such a complex structure evolve? In fact, much evidence indicates that bacterial flagella originated as simpler structures that were modified in a stepwise fashion over time. As in the case of the human eye (see Concept 25.6), biologists asked whether a less complex version of the flagellum could still benefit its owner. Analyses of hundreds of bacterial genomes indicate that only half of the flagellum's protein components appear to be necessary for it to function; the others are inessential or not encoded in the genomes of some species.

▼ **Figure 27.7 A prokaryotic flagellum.** The motor of a prokaryotic flagellum consists of a system of rings embedded in the cell wall and plasma membrane (TEM). The electron transport chain pumps protons out of the cell. The diffusion of protons back into the cell provides the force that turns a curved hook and thereby causes the attached filament to rotate and propel the cell. (This diagram shows flagellar structures characteristic of gram-negative bacteria.)



VISUAL SKILLS ► Predict which of the four protein rings shown in this diagram are likely hydrophobic. Explain your answer.



Video: Prokaryotic Flagella

Of the 21 proteins required by all species studied to date, 19 are modified versions of proteins that perform other tasks in bacteria. For example, a set of 10 proteins in the motor is homologous to 10 similar proteins in a secretory system found in bacteria. (A secretory system is a protein complex that enables a cell to produce and release certain macromolecules.) Two other proteins in the motor are homologous to proteins that function in ion transport. The proteins that comprise the rod, hook, and filament are all related to each other and are descended from an ancestral protein that formed a pilus-like tube. These findings suggest that the bacterial flagellum evolved as other proteins were added to an ancestral secretory system. This is an example of *exaptation*, the process in which structures originally adapted for one function take on new functions through descent with modification.

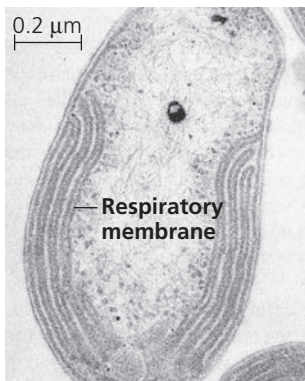
Internal Organization and DNA

The cells of prokaryotes are simpler than those of eukaryotes in both their internal structure and the physical arrangement of their DNA (see Figure 7.5). Prokaryotic cells lack the complex compartmentalization associated with the membrane-enclosed organelles found in eukaryotic cells. However, some prokaryotic cells do have specialized membranes that perform metabolic functions (Figure 27.8). These membranes are usually infoldings of the plasma membrane. Recent discoveries also indicate that some prokaryotes can store metabolic by-products in simple compartments that are made out of proteins; these compartments do not have a membrane.

The genome of a prokaryote is structurally different from a eukaryotic genome and in most cases has considerably less DNA. Prokaryotes generally have circular chromosomes (Figure 27.9), whereas eukaryotes have linear chromosomes. In addition, in prokaryotes the chromosome is associated with many fewer proteins than are the chromosomes

▼ Figure 27.8 Specialized membranes of prokaryotes.

(a) Infoldings of the plasma membrane, reminiscent of the cristae of mitochondria, function in cellular respiration in some aerobic prokaryotes (TEM). (b) Photosynthetic prokaryotes called cyanobacteria have thylakoid membranes, much like those in chloroplasts (TEM).

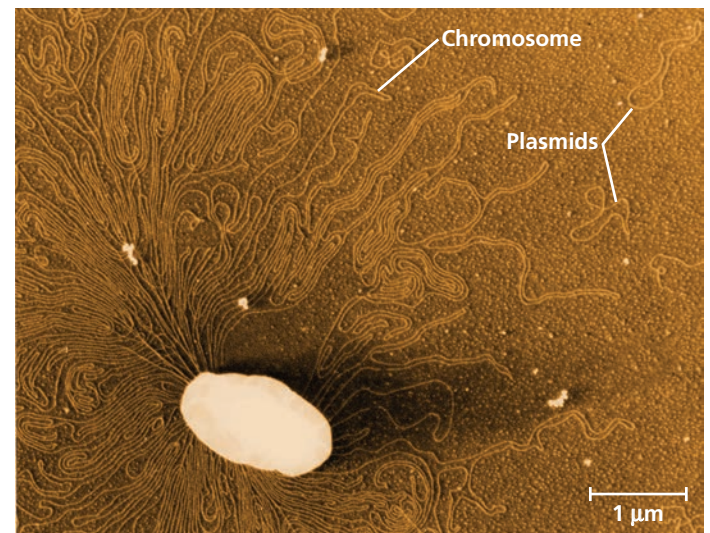


(a) Aerobic prokaryote



(b) Photosynthetic prokaryote

▼ **Figure 27.9 A prokaryotic chromosome and plasmids.** The thin, tangled loops surrounding this ruptured *Escherichia coli* cell are parts of the cell's large, circular chromosome (colorized TEM). Three of the cell's plasmids, the much smaller rings of DNA, are also shown.



of eukaryotes. Also unlike eukaryotes, prokaryotes lack a nucleus; their chromosome is located in the **nucleoid**, a region of cytoplasm that is not enclosed by a membrane. In addition to its single chromosome, a typical prokaryotic cell may also have much smaller rings of independently replicating DNA molecules called **plasmids** (see Figure 27.9), most carrying only a few genes.

Although DNA replication, transcription, and translation are fundamentally similar processes in prokaryotes and eukaryotes, some of the details are different (see Chapter 17). For example, prokaryotic ribosomes are slightly smaller than eukaryotic ribosomes and differ in their protein and RNA content. These differences allow certain antibiotics, such as erythromycin and tetracycline, to bind to ribosomes and block protein synthesis in prokaryotes but not in eukaryotes. As a result, people can use these antibiotics to kill or inhibit the growth of bacteria without harming themselves.

Reproduction

Many prokaryotes can reproduce quickly in favorable environments. By *binary fission* (see Figure 12.12), a single prokaryotic cell divides into 2 cells, which then divide into 4, 8, 16, and so on. Under optimal conditions, many prokaryotes can divide every 1–3 hours; some species can produce a new generation in only 20 minutes. At this rate, a single prokaryotic cell could give rise to a colony outweighing Earth in only two days!

In reality, of course, this does not occur. The cells eventually exhaust their nutrient supply, poison themselves with metabolic wastes, face competition from other microorganisms, or are consumed by other organisms. Still, many prokaryotic species' potential for rapid population growth

emphasizes three key features of their biology: *They are small, they reproduce by binary fission, and they often have short generation times.* As a result, prokaryotic populations can consist of many trillions of individuals—far more than populations of multicellular eukaryotes, such as plants or animals.

Animation: Structure and Reproduction of Bacteria

CONCEPT CHECK 27.1

1. Describe two adaptations that enable prokaryotes to survive in environments too harsh for other organisms.
2. Why are most of the pathogenic bacteria gram-negative?
3. **MAKE CONNECTIONS** > Suggest a hypothesis to explain why the thylakoid membranes of chloroplasts resemble those of cyanobacteria. Refer to Figures 7.18 and 22.21.

For suggested answers, see Appendix A.

CONCEPT 27.2

Rapid reproduction, mutation, and genetic recombination promote genetic diversity in prokaryotes

As we discussed in Unit Four, evolution cannot occur without genetic variation. The diverse adaptations exhibited by prokaryotes suggest that their populations must have considerable genetic variation—and they do. In this section, we'll examine three factors that give rise to high levels of genetic diversity in prokaryotes: rapid reproduction, mutation, and genetic recombination.

Rapid Reproduction and Mutation

In sexually reproducing species, the generation of a novel allele by a new mutation is rare for any particular gene. Instead, most of the genetic variation in sexual populations results from the way existing alleles are arranged in new combinations during meiosis and fertilization (see Concept 13.4). Prokaryotes do not reproduce sexually, so at first glance their extensive genetic variation may seem puzzling. But in many species, this variation can result from a combination of rapid reproduction and mutation.

Consider the bacterium *Escherichia coli* as it reproduces by binary fission in a human intestine, one of its natural environments. After repeated rounds of division, most of the offspring cells are genetically identical to the original parent cell. However, if errors occur during DNA replication, some of the offspring cells may differ genetically. The probability of such a mutation occurring in a given *E. coli* gene is about one in 10 million (1×10^{-7}) per cell division. But among the 2×10^{10} new *E. coli* cells that arise each day in a person's intestine, there will be approximately $(2 \times 10^{10}) \times (1 \times 10^{-7}) = 2,000$ bacteria that have a mutation in that gene. The total number

of new mutations when all 4,300 *E. coli* genes are considered is about $4,300 \times 2,000$ —more than 8 million per day per human host.

The key point is that new mutations, though rare on a per gene basis, can increase genetic diversity quickly in species with short generation times and large populations. This diversity, in turn, can lead to rapid evolution (**Figure 27.10**): Individuals that are genetically better equipped for their

▼ Figure 27.10

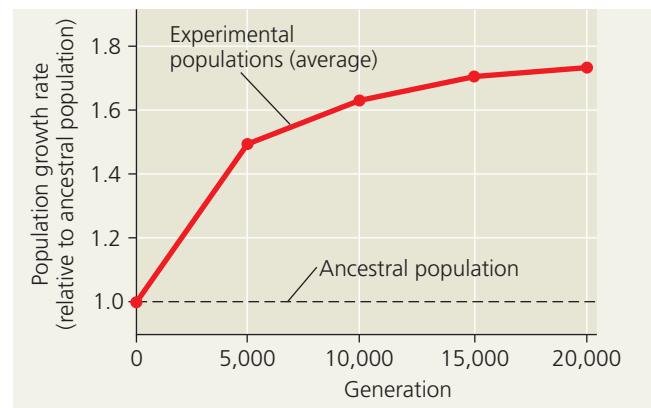
Inquiry Can prokaryotes evolve rapidly in response to environmental change?

Experiment Vaughn Cooper and Richard Lenski tested the ability of *E. coli* populations to adapt to a new environment. They established 12 populations, each founded by a single cell from an *E. coli* strain, and followed these populations for 20,000 generations (3,000 days).

To maintain a continual supply of resources, each day the researchers performed a *serial transfer*: They transferred 0.1 mL of each population to a new tube containing 9.9 mL of fresh growth medium. The growth medium used throughout the experiment provided a challenging environment that contained only low levels of glucose and other resources needed for growth.

Samples were periodically removed from the 12 populations and grown in competition with the common ancestral strain in the experimental (low-glucose) environment.

Results The fitness of the experimental populations, as measured by the growth rate of each population, increased rapidly for the first 5,000 generations (2 years) and more slowly for the next 15,000 generations. The graph shows the averages for the 12 populations.



Conclusion Populations of *E. coli* continued to accumulate beneficial mutations for 20,000 generations, allowing rapid evolution of increased population growth rates in their new environment.

Data from V. S. Cooper and R. E. Lenski, The population genetics of ecological specialization in evolving *Escherichia coli* populations, *Nature* 407:736–739 (2000).

WHAT IF? > Suggest possible functions of the genes whose sequence or expression was altered as the experimental populations evolved in the low-glucose environment.

environment tend to survive and reproduce at higher rates than other individuals. The ability of prokaryotes to adapt rapidly to new conditions highlights the point that although the structure of their cells is simpler than that of eukaryotic cells, prokaryotes are not “primitive” or “inferior” in an evolutionary sense. They are, in fact, highly evolved: For 3.5 billion years, prokaryotic populations have responded successfully to many types of environmental challenges.

Genetic Recombination

Although new mutations are a major source of variation in prokaryotic populations, additional diversity arises from *genetic recombination*, the combining of DNA from two sources. In eukaryotes, the sexual processes of meiosis and fertilization combine DNA from two individuals in a single zygote. But meiosis and fertilization do not occur in prokaryotes. Instead, three other mechanisms—transformation, transduction, and conjugation—can bring together prokaryotic DNA from different individuals (that is, different cells). When the individuals are members of different species, this movement of genes from one organism to another is called *horizontal gene transfer*. Although scientists have found evidence that each of these mechanisms can transfer DNA within and between species in both domain Bacteria and domain Archaea, to date most of our knowledge comes from research on bacteria.

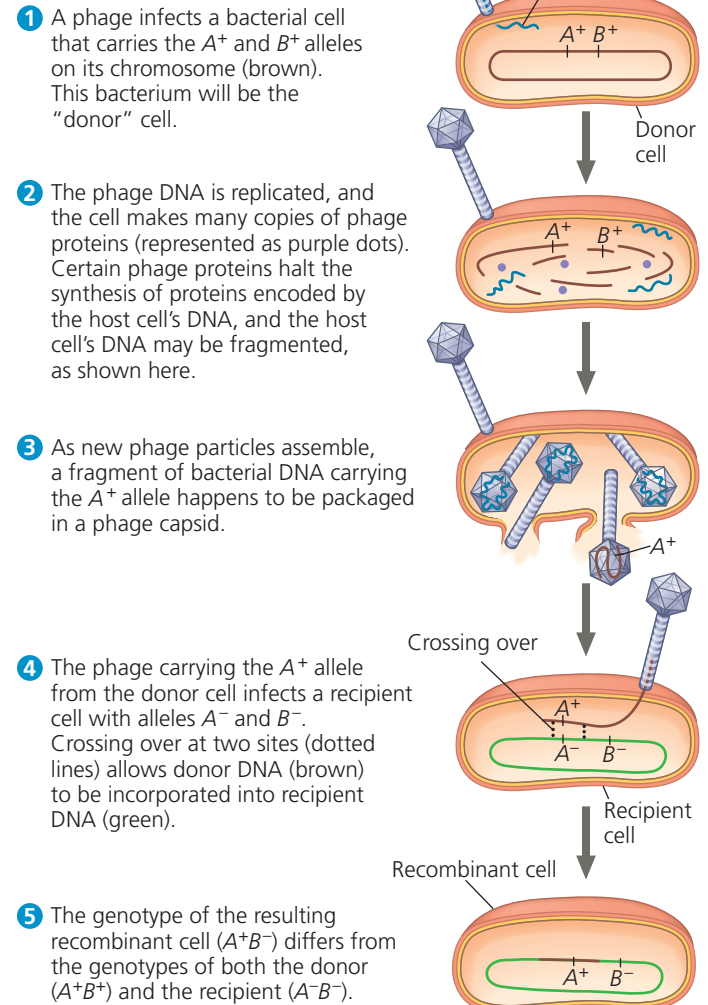
Transformation and Transduction

In **transformation**, the genotype and possibly phenotype of a prokaryotic cell are altered by the uptake of foreign DNA from its surroundings. For example, a harmless strain of *Streptococcus pneumoniae* can be transformed into pneumonia-causing cells if the cells are exposed to DNA from a pathogenic strain (see Concept 16.1). This transformation occurs when a nonpathogenic cell takes up a piece of DNA carrying the allele for pathogenicity and replaces its own allele with the foreign allele, an exchange of homologous DNA segments. The cell is now a recombinant: Its chromosome contains DNA derived from two different cells.

For many years after transformation was discovered in laboratory cultures, most biologists thought it was too rare and haphazard to play an important role in natural bacterial populations. But researchers have since learned that many bacteria have cell-surface proteins that recognize DNA from closely related species and transport it into the cell. Once inside the cell, the foreign DNA can be incorporated into the genome by homologous DNA exchange.

In **transduction**, phages (from “bacteriophages,” the viruses that infect bacteria) carry prokaryotic genes from one host cell to another. In most cases, transduction results from accidents that occur during the phage replicative cycle (**Figure 27.11**). A virus that carries prokaryotic DNA

▼ **Figure 27.11 Transduction.** Phages may carry pieces of a bacterial chromosome from one cell (the donor) to another (the recipient). If crossing over occurs after the transfer, genes from the donor may be incorporated into the recipient’s genome.



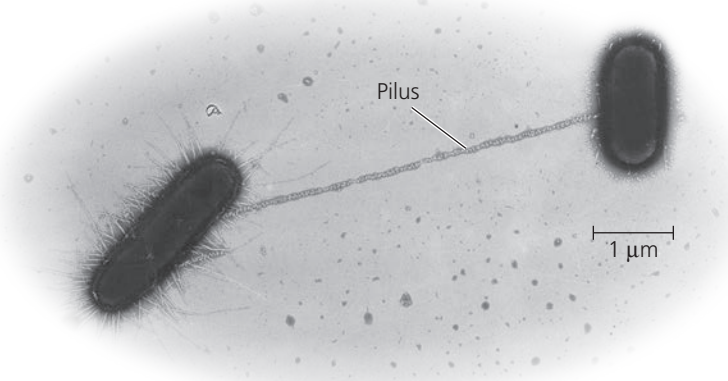
VISUAL SKILLS ► Based on this diagram, describe the circumstances in which a transduction event would result in horizontal gene transfer.

may not be able to replicate because it lacks some or all of its own genetic material. However, the virus can attach to another prokaryotic cell (a recipient) and inject prokaryotic DNA acquired from the first cell (the donor). If some of this DNA is then incorporated into the recipient cell’s chromosome by crossing over, a recombinant cell is formed.

Conjugation and Plasmids

In a process called **conjugation**, DNA is transferred between two prokaryotic cells (usually of the same species) that are temporarily joined. In bacteria, the DNA transfer is always one-way: One cell donates the DNA, and the other receives it. We’ll focus here on the mechanism used by *E. coli*.

▼ **Figure 27.12 Bacterial conjugation.** The *E. coli* donor cell (left) extends a pilus that attaches to a recipient cell, a key first step in the transfer of DNA. The pilus is a flexible tube of protein subunits (TEM).



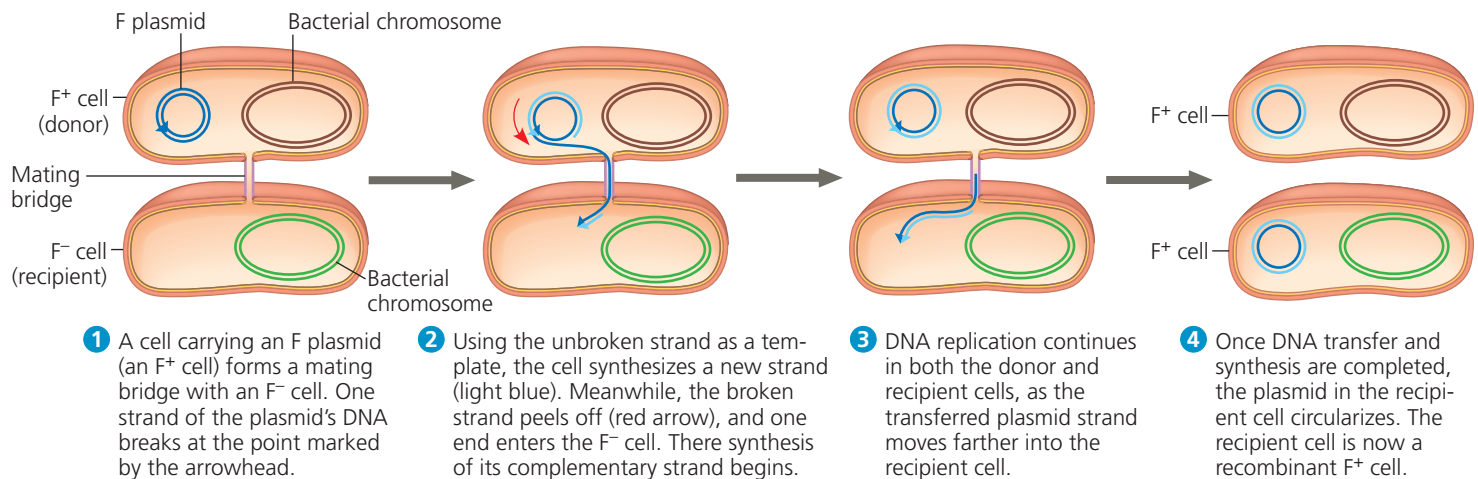
First, a pilus of the donor cell attaches to the recipient (Figure 27.12). The pilus then retracts, pulling the two cells together, like a grappling hook. The next step is thought to be the formation of a temporary structure between the two cells, a “mating bridge” through which the donor may transfer DNA to the recipient. However, the mechanism by which DNA transfer occurs is unclear; indeed, recent evidence indicates that DNA may pass directly through the hollow pilus.

The ability to form pili and donate DNA during conjugation results from the presence of a particular piece of DNA called the **F factor** (F for fertility). The F factor of *E. coli* consists of about 25 genes, most required for the production of pili. As shown in Figure 27.13, the F factor can exist either as a plasmid or as a segment of DNA within the bacterial chromosome.

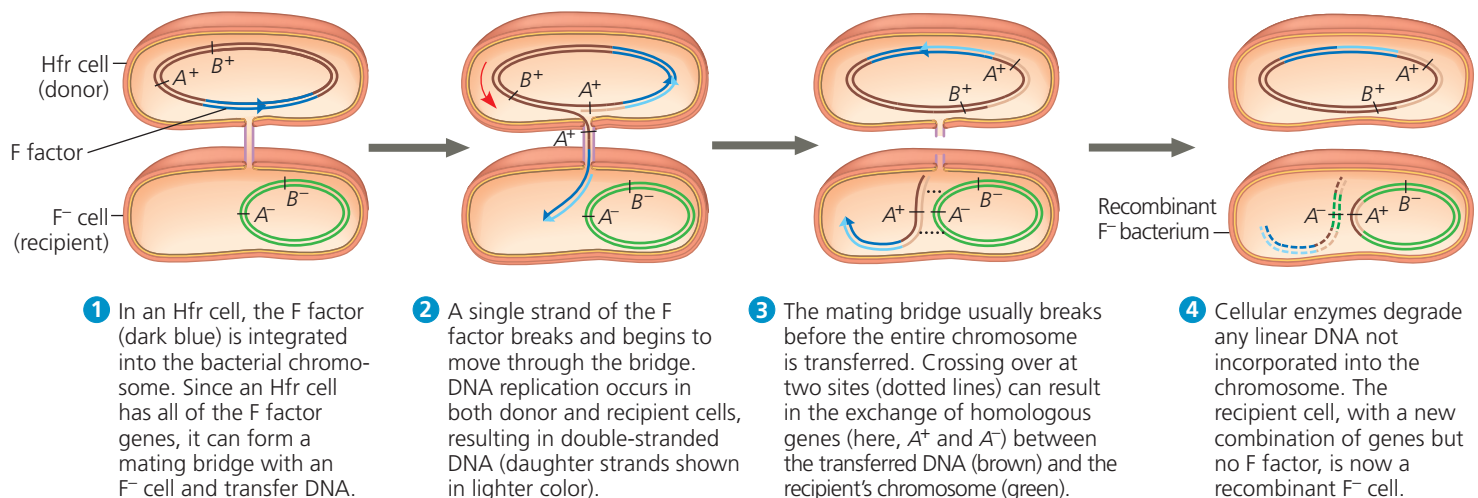
The F Factor as a Plasmid The F factor in its plasmid form is called the **F plasmid**. Cells containing the F plasmid,

▼ **Figure 27.13 Conjugation and recombination in *E. coli*.** The DNA replication that accompanies transfer of an F plasmid or part of an Hfr bacterial chromosome is called *rolling circle replication*. In effect, the intact circular parental DNA strand “rolls” as its other strand peels off and a new complementary strand is synthesized.

Figure Walkthrough



(a) Conjugation and transfer of an F plasmid



(b) Conjugation and transfer of part of an Hfr bacterial chromosome, resulting in recombination. A⁺/A⁻ and B⁺/B⁻ indicate alleles for gene A and gene B, respectively.

designated F^+ cells, function as DNA donors during conjugation (**Figure 27.13a**). Cells lacking the F factor, designated F^- , function as DNA recipients during conjugation. The F^+ condition is transferable in the sense that an F^+ cell converts an F^- cell to F^+ if a copy of the entire F plasmid is transferred. Even if this does not occur, as long as some of the F plasmid's DNA is transferred successfully to the recipient cell, that cell is now a recombinant cell.

The F Factor in the Chromosome Chromosomal genes can be transferred during conjugation when the donor cell's F factor is integrated into the chromosome. A cell with the F factor built into its chromosome is called an *Hfr cell* (for *high frequency of recombination*). Like an F^+ cell, an Hfr cell functions as a donor during conjugation with an F^- cell (**Figure 27.13b**). When chromosomal DNA from an Hfr cell enters an F^- cell, homologous regions of the Hfr and F^- chromosomes may align, allowing segments of their DNA to be exchanged. As a result, the recipient cell becomes a recombinant bacterium that has genes derived from the chromosomes of two different cells—a new genetic variant on which evolution can act.

R Plasmids and Antibiotic Resistance During the 1950s in Japan, physicians started noticing that some hospital patients with bacterial dysentery, which produces severe diarrhea, did not respond to antibiotics that had been effective in the past. Apparently, resistance to these antibiotics had evolved in some strains of *Shigella*, the bacterium that causes the disease.

Eventually, researchers began to identify the specific genes that confer antibiotic resistance in *Shigella* and other pathogenic bacteria. Sometimes mutation in a chromosomal gene of the pathogen can confer resistance. For example, a mutation in one gene may make it less likely that the pathogen will transport a particular antibiotic into its cell. Mutation in a different gene may alter the intracellular target protein for an antibiotic molecule, reducing its inhibitory effect. In other cases, bacteria have “resistance genes,” which code for enzymes that specifically destroy or otherwise hinder the effectiveness of certain antibiotics, such as tetracycline or ampicillin. Such resistance genes are often carried by plasmids known as **R plasmids** (R for *resistance*).

Exposing a bacterial population to a specific antibiotic will kill antibiotic-sensitive bacteria but not those that happen to have R plasmids with genes that counter the antibiotic. Under these circumstances, we would predict that natural selection would cause the fraction of the bacterial population carrying genes for antibiotic resistance to increase, and that is exactly what happens. The medical consequences are also predictable: Resistant strains of pathogens are becoming more common, making the treatment of certain bacterial infections more difficult. The problem is compounded by the fact that many R plasmids, like F plasmids, have genes that encode pili and enable DNA transfer from one bacterial cell to another by conjugation. Making the problem still worse, some R plasmids carry genes for resistance to as many as ten antibiotics.

CONCEPT CHECK 27.2

1. Although rare on a per gene basis, new mutations can add considerable genetic variation to prokaryotic populations in each generation. Explain how this occurs.
2. Distinguish between the three mechanisms by which bacteria can transfer DNA from one bacterial cell to another.
3. Why do F^- cells always serve as DNA recipients during the process of conjugation between two prokaryotic cells?
4. **WHAT IF? >** If a nonpathogenic bacterium were to acquire resistance to antibiotics, could this strain pose a health risk to people? In general, how does DNA transfer among bacteria affect the spread of resistance genes?

For suggested answers, see Appendix A.

CONCEPT 27.3

Diverse nutritional and metabolic adaptations have evolved in prokaryotes

The extensive genetic variation found in prokaryotes is reflected in their diverse nutritional adaptations. Like all organisms, prokaryotes can be categorized by how they obtain energy and the carbon used in building the organic molecules that make up cells. Every type of nutrition observed in eukaryotes is represented among prokaryotes, along with some nutritional modes unique to prokaryotes. In fact, prokaryotes have an astounding range of metabolic adaptations, much broader than that found in eukaryotes.

Organisms that obtain energy from light are called *phototrophs*, and those that obtain energy from chemicals are called *chemotrophs*. Organisms that need only CO_2 or related compounds as a carbon source are called *autotrophs*. In contrast, *heterotrophs* require at least one organic nutrient, such as glucose, to make other organic compounds. Combining possible energy sources and carbon sources results in four major modes of nutrition, summarized in **Table 27.1**.

The Role of Oxygen in Metabolism

Prokaryotic metabolism also varies with respect to oxygen (O_2). **Obligate aerobes** must use O_2 for cellular respiration and cannot grow without it. **Obligate anaerobes**, on the other hand, are poisoned by O_2 . Some obligate anaerobes live exclusively by fermentation; others extract chemical energy by **anaerobic respiration**, in which substances other than O_2 , such as nitrate ions (NO_3^-) or sulfate ions (SO_4^{2-}), accept electrons at the “downhill” end of electron transport chains. **Facultative anaerobes** use O_2 if it is present but can also carry out fermentation or anaerobic respiration in an anaerobic environment.

Nitrogen Metabolism

Nitrogen is essential for the production of amino acids and nucleic acids in all organisms. Whereas eukaryotes can obtain

Table 27.1 Major Nutritional Modes

Mode	Energy Source	Carbon Source	Types of Organisms
AUTOTROPH			
Photoautotroph	Light	CO ₂ , HCO ₃ ⁻ , or related compound	Photosynthetic prokaryotes (for example, cyanobacteria); plants; certain protists (for example, algae)
Chemoautotroph	Inorganic chemicals (such as H ₂ S, NH ₃ , or Fe ²⁺)	CO ₂ , HCO ₃ ⁻ , or related compound	Unique to certain prokaryotes (for example, <i>Sulfolobus</i>)
HETEROTROPH			
Photoheterotroph	Light	Organic compounds	Unique to certain aquatic and salt-loving prokaryotes (for example, <i>Rhodobacter</i> , <i>Chloroflexus</i>)
Chemoheterotroph	Organic compounds	Organic compounds	Many prokaryotes (for example, <i>Clostridium</i>) and protists; fungi; animals; some plants

nitrogen only from a limited group of nitrogen compounds, prokaryotes can metabolize nitrogen in many forms. For example, some cyanobacteria and some methanogens (a group of archaea) convert atmospheric nitrogen (N₂) to ammonia (NH₃), a process called **nitrogen fixation**. The cells can then incorporate this “fixed” nitrogen into amino acids and other organic molecules. In terms of nutrition, nitrogen-fixing cyanobacteria are some of the most self-sufficient organisms, since they need only light, CO₂, N₂, water, and some minerals to grow.

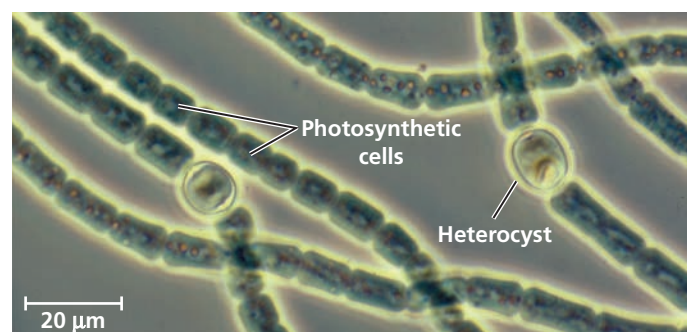
Nitrogen fixation has a large impact on other organisms. For example, nitrogen-fixing prokaryotes can increase the nitrogen available to plants, which cannot use atmospheric nitrogen but can use the nitrogen compounds that the prokaryotes produce from ammonia. Concept 55.4 discusses this and other essential roles that prokaryotes play in the nitrogen cycles of ecosystems.

Metabolic Cooperation

Cooperation between prokaryotic cells allows them to use environmental resources they could not use as individual cells. In some cases, this cooperation takes place between specialized cells of a filament. For instance, the cyanobacterium *Anabaena* has genes that encode proteins for photosynthesis and for nitrogen fixation. However, a single cell cannot carry out both processes at the same time because photosynthesis produces O₂, which inactivates the enzymes involved in nitrogen fixation. Instead of living as isolated cells, *Anabaena*

▼ Figure 27.14 Metabolic cooperation in a prokaryote.

In the filamentous freshwater cyanobacterium *Anabaena*, heterocysts fix nitrogen, while the other cells carry out photosynthesis (LM).



forms filamentous chains (Figure 27.14). Most cells in a filament carry out only photosynthesis, while a few specialized cells called **heterocysts** (sometimes called *heterocytes*) carry out only nitrogen fixation. Each heterocyst is surrounded by a thickened cell wall that restricts entry of O₂ produced by neighboring photosynthetic cells. Intercellular connections allow heterocysts to transport fixed nitrogen to neighboring cells and to receive carbohydrates.

Metabolic cooperation between different prokaryotic species often occurs in surface-coating colonies known as **biofilms**. Cells in a biofilm secrete signaling molecules that recruit nearby cells, causing the colonies to grow. The cells also produce polysaccharides and proteins that stick the cells to the substrate and to one another; these polysaccharides and proteins form the capsule, or slime layer, mentioned earlier in the chapter. Channels in the biofilm allow nutrients to reach cells in the interior and wastes to be expelled. Biofilms are common in nature, but they can cause problems by contaminating industrial products and medical equipment and contributing to tooth decay and more serious health problems. Altogether, damage caused by biofilms costs billions of dollars annually.

In another example of cooperation between prokaryotes, sulfate-consuming bacteria coexist with methane-consuming archaea in ball-shaped aggregates on the ocean floor. The bacteria appear to use the archaea’s waste products, such as organic compounds and hydrogen. In turn, the bacteria produce sulfur compounds that the archaea use as oxidizing agents when they consume methane in the absence of oxygen. This partnership has global ramifications: Each year, these archaea consume an estimated 300 billion kg of methane, a major greenhouse gas (see Concept 56.4).

CONCEPT CHECK 27.3

1. Distinguish between the four major modes of nutrition, noting which are unique to prokaryotes.
2. A bacterium requires only the amino acid methionine as an organic nutrient and lives in lightless caves. What mode of nutrition does it employ? Explain.
3. **WHAT IF? >** Describe what you might eat for a typical meal if humans, like cyanobacteria, could fix nitrogen.

For suggested answers, see Appendix A.

CONCEPT 27.4

Prokaryotes have radiated into a diverse set of lineages

Since their origin 3.5 billion years ago, prokaryotic populations have radiated extensively as a wide range of structural and metabolic adaptations have evolved in them. Collectively, these adaptations have enabled prokaryotes to inhabit every environment known to support life—if there are organisms in a particular place, some of those organisms are prokaryotes. Yet despite their obvious success, it is only in recent decades that advances in genomics have begun to reveal the full extent of prokaryotic diversity.

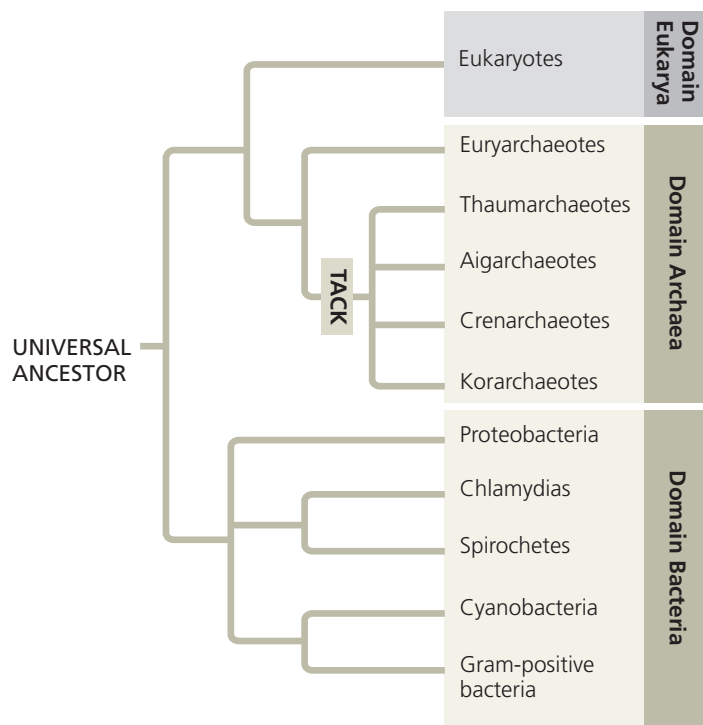
An Overview of Prokaryotic Diversity

In the 1970s, microbiologists began using small-subunit ribosomal RNA as a marker for evolutionary relationships. Their results indicated that many prokaryotes once classified as bacteria are actually more closely related to eukaryotes and belong in a domain of their own: Archaea. Microbiologists have since analyzed larger amounts of genetic data—including more than 1,700 entire genomes—and have concluded that a few traditional taxonomic groups, such as cyanobacteria, are monophyletic. However, other traditional groups, such as gram-negative bacteria, are scattered throughout several lineages. **Figure 27.15** shows one phylogenetic hypothesis for some of the major taxa of prokaryotes based on molecular systematics.

One lesson from studying prokaryotic phylogeny is that the genetic diversity of prokaryotes is immense. When researchers began to sequence the genes of prokaryotes, they could investigate only the small fraction of species that could be cultured in the laboratory. In the 1980s, researchers began using the polymerase chain reaction (PCR; see Figure 19.8) to analyze the genes of prokaryotes collected from the environment (such as from soil or water samples). Such “genetic prospecting” is now widely used; in fact, today entire prokaryotic genomes can be obtained from environmental samples using *metagenomics* (see Concept 20.1). Each year these techniques add new branches to the tree of life. While only about 10,600 prokaryotic species worldwide have been assigned scientific names, a single handful of soil could contain 10,000 prokaryotic species by some estimates. Taking full stock of this diversity will require many years of research.

Another important lesson from molecular systematics is that horizontal gene transfer has played a key role in the evolution of prokaryotes. Over hundreds of millions of years, prokaryotes have acquired genes from even distantly related species, and they continue to do so today. As a result, significant portions of the genomes of many prokaryotes are actually mosaics of genes imported from other species. For example, a study of 329 sequenced bacterial genomes found

▼ **Figure 27.15 A simplified phylogeny of prokaryotes.** This tree shows relationships among major prokaryotic groups based on molecular data; some of these relationships are shown as polytomies to reflect their uncertain order of divergence. Recent studies indicate that within Archaea, the thaumarchaeotes, aigarchaeotes, crenarchaeotes, and korarchaeotes are closely related; systematists have placed them in a supergroup called “TACK” in reference to the first letters of their names.



VISUAL SKILLS ► Based on this phylogenetic tree diagram, which domain is the sister group of Archaea?

that an average of 75% of the genes in each genome had been transferred horizontally at some point in their evolutionary history. As we saw in Concept 22.6, such gene transfers can make it difficult to determine phylogenetic relationships. Still, it is clear that for billions of years, the prokaryotes have evolved in two separate lineages, the bacteria and the archaea (see Figure 27.15).

Bacteria

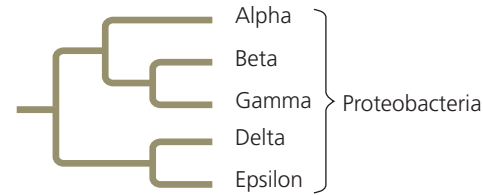


As surveyed in **Figure 27.16**, bacteria include the vast majority of prokaryotic species familiar to most people, from the pathogenic species that cause strep throat and tuberculosis to the beneficial species used to make Swiss cheese and yogurt. Every major mode of nutrition and metabolism is represented among bacteria, and even a small taxonomic group of bacteria may contain species exhibiting many different nutritional modes. As we'll see, the diverse nutritional and metabolic capabilities of bacteria—and archaea—are behind the great impact these organisms have on Earth and its life.

▼ Figure 27.16 Exploring Selected Major Groups of Bacteria

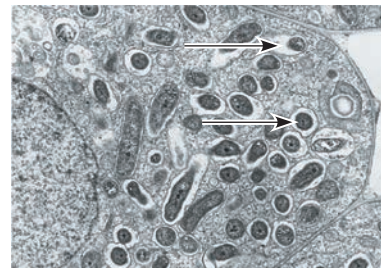
Proteobacteria

This large and diverse clade of gram-negative bacteria includes photoautotrophs, chemoautotrophs, and heterotrophs. Some are anaerobic, while others are aerobic. Molecular systematists currently recognize five subgroups of proteobacteria; the phylogenetic tree at right shows their relationships based on molecular data.



Subgroup: Alpha Proteobacteria

Many of the species in this subgroup are closely associated with eukaryotic hosts. For example, *Rhizobium* species live in nodules within the roots of legumes (plants of the pea/bean family), where the bacteria convert atmospheric N_2 to compounds the host plant can use to make proteins. Species in the genus *Agrobacterium* produce tumors in plants; genetic engineers use these bacteria to carry foreign DNA into the genomes of crop plants. Scientists hypothesize that mitochondria evolved from aerobic alpha proteobacteria through endosymbiosis.



Rhizobium (arrows) inside a root cell of a legume (TEM)

2.5 μm

Subgroup: Beta Proteobacteria

This nutritionally diverse subgroup includes *Nitrosomonas*, a genus of soil bacteria that play an important role in nitrogen recycling by oxidizing ammonium (NH_4^+), producing nitrite (NO_2^-) as a waste product. Other members of this subgroup include a wide range of aquatic species, such as the photoheterotroph *Rubrivivax*, along with pathogens such as the species that causes the sexually transmitted disease gonorrhea, *Neisseria gonorrhoeae*.

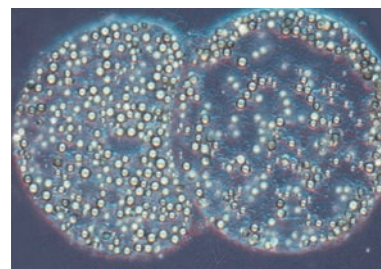


Nitrosomonas (colorized TEM)

1 μm

Subgroup: Gamma Proteobacteria

This subgroup's autotrophic members include sulfur bacteria, such as *Thiomargarita namibiensis*, which obtain energy by oxidizing H_2S , producing sulfur as a waste product (the small globules in the photograph at right). Some heterotrophic gamma proteobacteria are pathogens; for example, *Legionella* causes Legionnaires' disease, *Salmonella* is responsible for some cases of food poisoning, and *Vibrio cholerae* causes cholera. *Escherichia coli*, a common resident of the intestines of humans and other mammals, normally is not pathogenic.



Thiomargarita namibiensis containing sulfur wastes (LM)

200 μm

Subgroup: Delta Proteobacteria

This subgroup includes the slime-secreting myxobacteria. When the soil dries out or food is scarce, the cells congregate into a fruiting body that releases resistant "myxospores." These cells found new colonies in favorable environments. Another group of delta proteobacteria, the bdellovibrios, attack other bacteria, charging at up to 100 $\mu m/sec$ (comparable to a human running 240 km/hr). The attack begins when a bdellovibrio attaches to specific molecules found on the outer covering of some bacterial species. The bdellovibrio then drills into its prey by using digestive enzymes and spinning at 100 revolutions per second.



Fruiting bodies of *Chondromyces crocatus*, a myxobacterium (SEM)

300 μm

Subgroup: Epsilon Proteobacteria

Most species in this subgroup are pathogenic to humans or other animals. Epsilon proteobacteria include *Campylobacter*, which causes blood poisoning and intestinal inflammation, and *Helicobacter pylori*, which causes stomach ulcers.



Helicobacter pylori (colorized TEM)

2 μm

Table 27.2 A Comparison of the Three Domains of Life

CHARACTERISTIC	DOMAIN		
	Bacteria	Archaea	Eukarya
Nuclear envelope	Absent	Absent	Present
Membrane-enclosed organelles	Absent	Absent	Present
Peptidoglycan in cell wall	Present	Absent	Absent
Membrane lipids	Unbranched hydrocarbons	Some branched hydrocarbons	Unbranched hydrocarbons
RNA polymerase	One kind	Several kinds	Several kinds
Initiator amino acid for protein synthesis	Formyl-methionine	Methionine	Methionine
Introns in genes	Very rare	Present in some genes	Present in many genes
Response to the antibiotics streptomycin and chloramphenicol	Growth usually inhibited	Growth not inhibited	Growth not inhibited
Histones associated with DNA	Absent	Present in some species	Present
Circular chromosome	Present	Present	Absent
Growth at temperatures > 100°C	No	Some species	No

Archaea



Archaea share certain traits with bacteria and other traits with eukaryotes (**Table 27.2**).

However, archaea also have many unique characteristics, as we would expect in a taxon that has followed a separate evolutionary path for so long.

The first prokaryotes assigned to domain Archaea live in environments so extreme that few other organisms can survive there. Such organisms are called **extremophiles**, meaning “lovers” of extreme conditions (from the Greek *philos*, lover), and include extreme halophiles and extreme thermophiles.

Extreme halophiles (from the Greek *halo*, salt) live in highly saline environments, such as the Great Salt Lake in Utah, the Dead Sea in Israel, and the Spanish lake shown in Figure 27.1. Some species merely tolerate salinity, while others require an environment that is several times saltier than seawater (which has a salinity of 3.5%). For example, the proteins and cell wall of *Halobacterium* have unusual features that improve function in extremely salty environments but render these organisms incapable of survival if the salinity drops below 9%.



Figure 27.17 Extreme thermophiles. Thermophilic prokaryotes grow in the hot water of geysers in the Valley of Geysers in Kamchatka, Russia.

MAKE CONNECTIONS > How might the enzymes of thermophiles differ from those of other organisms? (Review enzymes in Concept 6.4.)

Extreme thermophiles (from the Greek *thermos*, hot) thrive in very hot environments (**Figure 27.17**). For example, archaea in the genus *Sulfolobus* live in sulfur-rich volcanic springs as hot as 90°C. At temperatures this high, the cells of most organisms die because their DNA does not remain in a double helix and many of their proteins denature. *Sulfolobus* and other extreme thermophiles avoid this fate because they have structural and biochemical adaptations that make their DNA and proteins stable at high temperatures. One extreme thermophile that lives near deep-sea hot springs called *hydrothermal vents* is informally known as “strain 121,” since it can reproduce even at 121°C. Another extreme thermophile, *Pyrococcus furiosus*, is used in biotechnology as a source of DNA polymerase for the PCR technique (see Figure 19.8).

Many other archaea live in more moderate environments. Consider the **methanogens**, archaea that release methane as a by-product of their unique ways of obtaining energy. Many methanogens use CO₂ to oxidize H₂, a process that produces both energy and methane waste. Among the strictest of anaerobes, methanogens are poisoned by O₂. Although some methanogens live in extreme environments, such as under kilometers of ice in Greenland, others live in swamps and marshes where other microorganisms have consumed all the O₂. The “marsh gas” found in such environments is the methane released by these archaea. Other species inhabit the anaerobic guts of cattle, termites, and other herbivores, playing an essential role in the nutrition of these animals. Methanogens are also useful to humans as decomposers in sewage treatment facilities.

Many extreme halophiles and all known methanogens are archaea in the clade Euryarchaeota (from the Greek *eury*, broad, a reference to their wide habitat range). The

euryarchaeotes also include some extreme thermophiles, though most thermophilic species belong to a second clade, Crenarchaeota (*cren* means “spring,” such as a hydrothermal spring). Metagenomic studies have identified many species of euryarchaeotes and crenarchaeotes that are not extremophiles. These archaea exist in habitats ranging from farm soils to lake sediments to the surface of the open ocean.

New findings continue to inform our understanding of archaeal phylogeny. For example, recent metagenomic studies have uncovered the genomes of many species that are not members of Euryarchaeota or Crenarchaeota. Moreover, phylogenomic analyses show that three of these newly discovered groups—the Thaumarchaeota, Aigarchaeota, and Korarchaeota—are more closely related to the Crenarchaeota than they are to the Euryarchaeota. These findings have led to the identification of a “supergroup” that contains the Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota (see Figure 27.15). This supergroup is referred to as “TACK” based on the names of the groups it includes. In 2015, the importance of the TACK supergroup was highlighted by the discovery of the Lokiarchaeotes, a group that is closely related to TACK archaea and that could possibly represent the long sought-after sister group of the eukaryotes. As such, the characteristics of Lokiarchaeotes may shed light on one of the major puzzles of biology today—how eukaryotes arose from their prokaryotic ancestors. The pace of these and other recent discoveries suggests that as metagenomic prospecting continues, the tree in Figure 27.15 will likely undergo further changes.

CONCEPT CHECK 27.4

1. Explain how molecular systematics and metagenomics have contributed to our understanding of the phylogeny and evolution of prokaryotes.
2. **WHAT IF? >** What would the discovery of a bacterial species that is a methanogen imply about the evolution of the methane-producing pathway?

For suggested answers, see Appendix A.

CONCEPT 27.5

Prokaryotes play crucial roles in the biosphere

If people were to disappear from the planet tomorrow, life on Earth would change for many species, but few would be driven to extinction. In contrast, prokaryotes are so important to the biosphere that if they were to disappear, the prospects of survival for many other species would be dim.

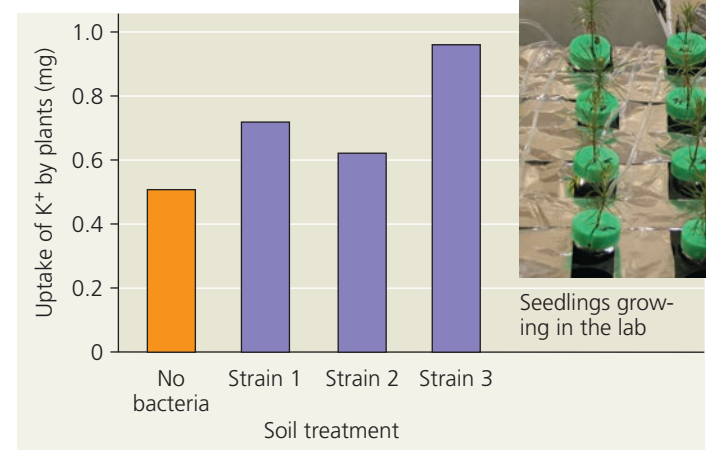
Chemical Recycling

The atoms that make up the organic molecules in all living things were at one time part of inorganic substances in the soil, air, and water. Sooner or later, those atoms will return to the

nonliving environment. Ecosystems depend on the continual recycling of chemical elements between the living and nonliving components of the environment, and prokaryotes play a major role in this process. For example, some chemoheterotrophic prokaryotes function as **decomposers**, breaking down dead organisms as well as waste products and thereby unlocking supplies of carbon, nitrogen, and other elements. Without the actions of prokaryotes and other decomposers such as fungi, life as we know it would cease. (See Concept 55.4 for a detailed discussion of chemical cycles.)

Prokaryotes also convert some molecules to forms that can be taken up by other organisms. Cyanobacteria and other autotrophic prokaryotes use CO₂ to make organic compounds such as sugars, which are then passed up through food chains. Cyanobacteria also produce atmospheric O₂, and a variety of prokaryotes fix atmospheric nitrogen (N₂) into forms that other organisms can use to make the building blocks of proteins and nucleic acids. Under some conditions, prokaryotes can increase the availability of nutrients that plants require for growth, such as nitrogen, phosphorus, and potassium (**Figure 27.18**). Prokaryotes can also *decrease* the availability of key plant nutrients; this occurs when prokaryotes “immobilize” nutrients by using them to synthesize molecules that remain within their cells. Thus, prokaryotes can have complex effects on soil nutrient concentrations. In marine environments, an archaean from the clade Crenarchaeota can perform nitrification, a key step in the nitrogen cycle (see Figure 55.14). Crenarchaeotes dominate the oceans by numbers, comprising an estimated 10²⁸ cells. The sheer abundance of these organisms suggests that they may have a large impact on the global nitrogen cycle.

✓ Figure 27.18 Impact of bacteria on soil nutrient availability. Pine seedlings grown in sterile soils to which one of three strains of the bacterium *Burkholderia glathei* had been added absorbed more potassium (K⁺) than did seedlings grown in soil without any bacteria. Other results (not shown) demonstrated that strain 3 increased the amount of K⁺ released from mineral crystals to the soil.



WHAT IF? > Estimate the average uptake of K⁺ for seedlings in soils with bacteria. What would you expect this average to be if bacteria had no effect on nutrient availability?



▲ Figure 27.19 Mutualism: bacterial “headlights.” The glowing oval below the eye of the flashlight fish (*Photoblepharon palpebratus*) is an organ harboring bioluminescent bacteria. The fish uses the light to attract prey and to signal potential mates. The bacteria receive nutrients from the fish.

Ecological Interactions

Prokaryotes play a central role in many ecological interactions. Consider **symbiosis** (from a Greek word meaning “living together”), an ecological relationship in which two species live in close contact with each other. Prokaryotes often form symbiotic associations with much larger organisms. In general, the larger organism in a symbiotic relationship is known as the **host**, and the smaller is known as the **symbiont**. There are many cases in which a prokaryote and its host participate in **mutualism**, an ecological interaction between two species in which both benefit (**Figure 27.19**). Other interactions take the form of **commensalism**, an ecological relationship in which one species benefits while the other is not harmed or helped in any significant way. For example, more than 150 bacterial species live on the outer surface of your body, covering portions of your skin with up to 10 million cells per square centimeter. Some of these species are commensalists: You provide them with food, such as the oils that exude from your pores, and a place to live, while they neither harm nor benefit you. Finally, some prokaryotes engage in **parasitism**, an ecological relationship in which a **parasite** eats the cell contents, tissues, or body fluids of its host. As a group, parasites harm but usually do not kill their host, at least not immediately (unlike a predator). Parasites that cause disease are known as **pathogens**, many of which are prokaryotic. (We’ll discuss mutualism, commensalism, and parasitism in greater detail in Concept 54.1.)

The very existence of an ecosystem can depend on prokaryotes. For example, consider the diverse ecological communities found at hydrothermal vents. These communities are densely populated by many different kinds of animals, including worms, clams, crabs, and fishes. But since sunlight does not penetrate to the deep ocean floor, the community does not include photosynthetic organisms. Instead, the energy that supports the community is derived from the metabolic activities of chemoautotrophic bacteria. These

bacteria harvest chemical energy from compounds such as hydrogen sulfide (H_2S) that are released from the vent. An active hydrothermal vent may support hundreds of eukaryotic species, but when the vent stops releasing chemicals, the chemoautotrophic bacteria cannot survive. As a result, the entire vent community collapses.

CONCEPT CHECK 27.5

1. Explain how prokaryotes, though small, can be considered giants in their collective impact on Earth and its life.
2. **MAKE CONNECTIONS** ▶ Review Figure 11.6. Then summarize the main steps by which cyanobacteria produce O_2 and use CO_2 to make organic compounds.

For suggested answers, see Appendix A.

CONCEPT 27.6

Prokaryotes have both beneficial and harmful impacts on humans

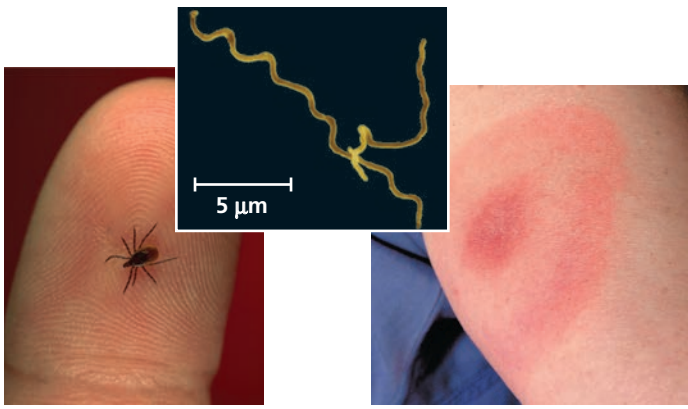
Although the best-known prokaryotes tend to be the bacteria that cause human illness, these pathogens represent only a small fraction of prokaryotic species. Many other prokaryotes have positive interactions with people, and some play essential roles in agriculture and industry.

Mutualistic Bacteria

As is true for many other eukaryotes, human well-being can depend on mutualistic prokaryotes. For example, our intestines are home to an estimated 500–1,000 species of bacteria; their cells outnumber all human cells in the body by a factor of ten. Different species live in different portions of the intestines, and they vary in their ability to process different foods. Many of these species are mutualists, digesting food that our own intestines cannot break down. The genome of one of these gut mutualists, *Bacteroides thetaiotaomicron*, includes a large array of genes involved in synthesizing carbohydrates, vitamins, and other nutrients needed by humans. Signals from the bacterium activate human genes that build the network of intestinal blood vessels necessary to absorb nutrient molecules. Other signals induce human cells to produce antimicrobial compounds to which *B. thetaiotaomicron* is not susceptible. This action may reduce the population sizes of other, competing species, thus potentially benefiting both *B. thetaiotaomicron* and its human host.

Pathogenic Bacteria

All the pathogenic prokaryotes known to date are bacteria, and they deserve their negative reputation. Bacteria cause about half of all human diseases. For example, more than 1 million people die each year of the lung disease tuberculosis, caused by *Mycobacterium tuberculosis*. And another



▲ **Figure 27.20 Lyme disease.** Ticks in the genus *Ixodes* spread the disease by transmitting the spirochete *Borrelia burgdorferi* (colorized SEM). A rash may develop at the site of the tick's bite; the rash may be large and ring-shaped (as shown) or much less distinctive.

2 million people die each year from diarrheal diseases caused by various bacteria.

Some bacterial diseases are transmitted by other species, such as fleas or ticks. In the United States, the most widespread pest-carried disease is Lyme disease, which infects 15,000 to 20,000 people each year (**Figure 27.20**). Caused by a bacterium carried by ticks that live on deer and field mice, Lyme disease can result in debilitating arthritis, heart disease, nervous disorders, and death if untreated.

Pathogenic prokaryotes usually cause illness by producing poisons, which are classified as exotoxins or endotoxins.

Exotoxins are proteins secreted by certain bacteria and other organisms. Cholera, a dangerous diarrheal disease, is caused by an exotoxin secreted by the proteobacterium *Vibrio cholerae*. The exotoxin stimulates intestinal cells to release chloride ions into the gut, and water follows by osmosis. In another example, the potentially fatal disease botulism is caused by botulinum toxin, an exotoxin secreted by the gram-positive bacterium *Clostridium botulinum* as it ferments various foods, including improperly canned meat, seafood, and vegetables. Like other exotoxins, the botulinum toxin can produce disease even if the bacteria that manufacture it are no longer present when the food is eaten. Another species in the same genus, *C. difficile*, produces exotoxins that cause severe diarrhea, resulting in more than 12,000 deaths per year in the United States alone.

Endotoxins are lipopolysaccharide components of the outer membrane of gram-negative bacteria. In contrast to exotoxins, endotoxins are released only when the bacteria die and their cell walls break down. Endotoxin-producing bacteria include species in the genus *Salmonella*, such as *Salmonella typhi*, which causes typhoid fever. You might have heard of food poisoning caused by other *Salmonella* species that can be found in poultry and some fruits and vegetables.

Since the 19th century, improved sanitation systems in the industrialized world have greatly reduced the threat of pathogenic bacteria. Antibiotics have saved a great many lives and reduced the incidence of disease. However, resistance to

antibiotics is currently evolving in many bacterial strains. As you read earlier, the rapid reproduction of bacteria enables cells carrying resistance genes to quickly give rise to large populations as a result of natural selection, and these genes can also spread to other species by horizontal gene transfer.

Horizontal gene transfer can also spread genes associated with virulence, turning normally harmless bacteria into potent pathogens. *E. coli*, for instance, is ordinarily a harmless symbiont in the human intestines, but pathogenic strains that cause bloody diarrhea have emerged. One of the most dangerous strains, O157:H7, is a global threat; in the United States alone, there are 75,000 cases of O157:H7 infection per year, often from contaminated beef or produce. Scientists have sequenced the genome of O157:H7 and compared it with the genome of a harmless strain of *E. coli* called K-12. They discovered that 1,387 out of the 5,416 genes in O157:H7 have no counterpart in K-12. Many of these 1,387 genes are found in chromosomal regions that include phage DNA. This suggests that at least some of the 1,387 genes were incorporated into the genome of O157:H7 through phage-mediated horizontal gene transfer (transduction). Some of the genes found only in O157:H7 are associated with virulence, including genes that code for adhesive fimbriae that enable O157:H7 to attach itself to the intestinal wall and extract nutrients.

Prokaryotes in Research and Technology

On a positive note, we reap many benefits from the metabolic capabilities of both bacteria and archaea. For example, people have long used bacteria to convert milk to cheese and yogurt. Bacteria are also used in the production of beer and wine, pepperoni, fermented cabbage (sauerkraut), and soy sauce. In recent decades, our greater understanding of prokaryotes has led to an explosion of new applications in biotechnology. Examples include the use of *E. coli* in gene cloning (see Figure 19.4) and the use of DNA polymerase from *Pyrococcus furiosus* in the PCR technique (see Figure 19.8). Through genetic engineering, we can modify bacteria to produce vitamins, antibiotics, hormones, and other products (see Concept 19.1). In addition, naturally occurring soil bacteria have potential as sources of new antibiotics, as you can explore in the **Scientific Skills Exercise**.

Recently, the prokaryotic CRISPR-Cas system, which helps bacteria and archaea defend against attack by viruses (see Figure 26.7), has been developed into a powerful new tool for altering genes in virtually any organism. The genomes of many prokaryotes contain short DNA repeats, called CRISPRs, that interact with proteins known as the Cas (CRISPR-associated) proteins. Cas proteins, acting together with “guide RNA” made from the CRISPR region, can cut any DNA sequence to which they are directed. Scientists have been able to exploit this system by introducing a Cas protein (Cas9) to guide RNA into cells whose DNA they want to alter (see Figure 19.14). Among

SCIENTIFIC SKILLS EXERCISE

Calculating and Interpreting Means and Standard Errors

Can Antibiotics Obtained from Soil Bacteria Help Fight Drug-Resistant Bacteria? Soil bacteria synthesize antibiotics, which they use against species that attack or compete with them. To date, these species have been inaccessible as sources for new medicines because 99% of soil bacteria cannot be grown using standard laboratory techniques. To address this problem, researchers developed a method in which soil bacteria grow in a simulated version of their natural environment; this led to the discovery of a new antibiotic, teixobactin. In this exercise, you'll calculate means and standard errors from an experiment that tested teixobactin's effectiveness against MRSA (methicillin-resistant *Staphylococcus aureus*; see Figure 21.14).

How the Experiment Was Done Researchers drilled tiny holes into a small plastic chip and filled the holes with a dilute aqueous solution containing soil bacteria and agar. The dilution had been calibrated so that only one bacterium was likely to grow in each hole. After the agar solidified, the chip was then placed in a container containing the original soil; nutrients and other essential materials from the soil diffused into the agar, allowing the bacteria to grow.

After isolating teixobactin from a soil bacterium, researchers performed the following experiment: mice infected with MRSA were given low (1 mg/kg) or high (5 mg/kg) doses of teixobactin or vancomycin, an existing antibiotic; in the control, mice infected with MRSA were not given an antibiotic. After 26 hours, researchers sampled infected mice and estimated the number of *S. aureus* colonies in each sample. Results were reported on a log scale; note that a decrease of 1.0 on this scale reflects a 10-fold decrease in MRSA abundance.

Data from the Experiment

Treatment	Dose (mg/kg)	Log of Number of Colonies	Mean ($\bar{x}$)
Control	—	9.0, 9.5, 9.0, 8.9	
Vancomycin	1.0	8.5, 8.4, 8.2	
	5.0	5.3, 5.9, 4.7	
Teixobactin	1.0	8.5, 6.0, 8.4, 6.0	
	5.0	3.8, 4.9, 5.2, 4.9	

► Plastic chip used to grow soil bacteria



Data from L. Ling et al. A new antibiotic kills pathogens without detectable resistance, *Nature* 517:455–459 (2015).

INTERPRET THE DATA

- The mean ($\bar{x}$) of a variable is the sum of the data values divided by the number of observations (n):

$$\bar{x} = \frac{\sum x_i}{n}$$

In this formula, x_i is the value of the i th observation of the variable; the $\sum$ symbol indicates that the n values of x are to be added together. Calculate the mean for each treatment.

- Use your results from question 1 to evaluate the effectiveness of vancomycin and teixobactin.
- The variation found in a set of data can be estimated by the standard deviation, s :

$$s = \sqrt{\frac{1}{n-1} \sum (x_i - \bar{x})^2}$$

Calculate the standard deviation for each treatment.

- The standard error (SE), which indicates how greatly the mean would likely vary if the experiment was repeated, is calculated as:

$$SE = \frac{s}{\sqrt{n}}$$

As a rough rule of thumb, if an experiment were to be repeated, the new mean typically would lie within two standard errors of the original mean (that is, within the range $\bar{x} \pm 2SE$). Calculate $\bar{x} \pm 2SE$ for each treatment, determine whether these ranges overlap, and interpret your results.

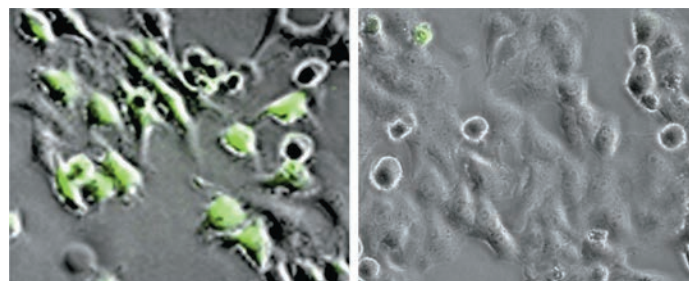


Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

other applications, this **CRISPR-Cas9 system** has already opened new lines of research on HIV, the virus that causes AIDS (**Figure 27.21**). While the CRISPR-Cas9 system can potentially be used in many different ways, care must be taken to guard against the unintended consequences that could arise when applying such a new and powerful technology.

Another valuable application of bacteria is to reduce our use of petroleum. Consider the plastics industry. Globally, each year about 350 billion pounds of plastic are produced from petroleum and used to make toys, storage containers, soft drink bottles, and many other items. These products degrade slowly, creating environmental problems. Bacteria produce natural plastics (**Figure 27.22**). For example, some bacteria synthesize a type of polymer known as PHA (polyhydroxyalkanoate), which they use to store chemical energy. The PHA can be extracted, formed into pellets, and used to make durable, yet biodegradable, plastics.

▼ **Figure 27.21 CRISPR: Opening new avenues of research for treating HIV infection.** (a) In laboratory experiments, untreated (control) human cells were susceptible to infection by HIV, the virus that causes AIDS. (b) In contrast, cells treated with a CRISPR-Cas9 system that targets HIV were resistant to viral infection. The CRISPR-Cas9 system was also able to remove HIV proviruses that had become incorporated into the DNA of human cells.

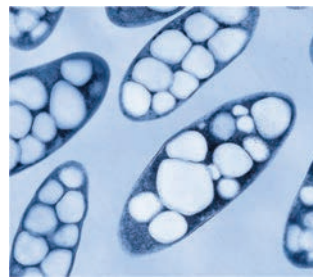


(a) **Control cells.** The green color indicates infection by HIV. (b) **Experimental cells.** These cells were treated with a CRISPR-Cas9 system that targets HIV.

Researchers are also seeking to reduce the use of petroleum and other fossil fuels by engineering bacteria that can produce ethanol from various forms of biomass, including agricultural waste, switchgrass, and corn.

Another way to harness prokaryotes is **bioremediation**, the use of organisms to remove pollutants from soil, air, or water. For example, anaerobic bacteria and archaea decompose the organic matter in sewage, converting it to material that can be used as landfill or fertilizer after chemical sterilization. Other bioremediation applications include cleaning up oil spills (**Figure 27.23**) and precipitating radioactive material (such as uranium) out of groundwater.

The usefulness of prokaryotes largely derives from their diverse forms of nutrition and metabolism. All this metabolic versatility evolved prior to the appearance of the structural novelties that heralded the evolution of eukaryotic organisms, discussed in the rest of this unit.



▲ **Figure 27.22** Bacteria synthesizing and storing **PHA**, a component of biodegradable plastics.

► **Figure 27.23**

Bioremediation of an oil spill.

Spraying fertilizer stimulates the growth of native bacteria that metabolize oil, increasing the speed of the breakdown process up to fivefold.



CONCEPT CHECK 27.6

1. Identify at least two ways that prokaryotes have affected you positively today.
2. A pathogenic bacterium's toxin causes symptoms that increase the bacterium's chance of spreading from host to host. Does this information indicate whether the poison is an exotoxin or endotoxin? Explain.
3. **WHAT IF? ►** How might a sudden and dramatic change in your diet affect the diversity of prokaryotic species that live in your digestive tract?

For suggested answers, see Appendix A.

27 Chapter Review

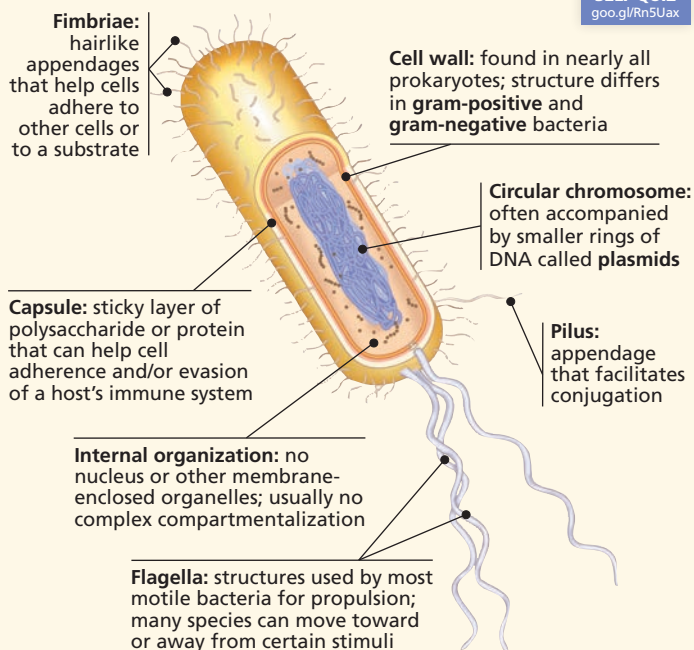
SUMMARY OF KEY CONCEPTS

CONCEPT 27.1

Structural and functional adaptations contribute to prokaryotic success (pp. 626–630)



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax



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- Many prokaryotic species can reproduce quickly by binary fission, leading to the formation of extremely large populations.



Describe features of prokaryotes that enable them to thrive in a wide range of different environments.

CONCEPT 27.2

Rapid reproduction, mutation, and genetic recombination promote genetic diversity in prokaryotes (pp. 630–633)

- Because prokaryotes can often proliferate rapidly, mutations can quickly increase a population's genetic variation. As a result, prokaryotic populations often can evolve in short periods of time in response to changing conditions.
- Genetic diversity in prokaryotes also can arise by recombination of the DNA from two different cells (via transformation, transduction, or conjugation). By transferring advantageous alleles, such as ones for antibiotic resistance, recombination can promote adaptive evolution in prokaryotic populations.



Mutations are rare and prokaryotes reproduce asexually, yet their populations can have high genetic diversity. Explain how this can occur.

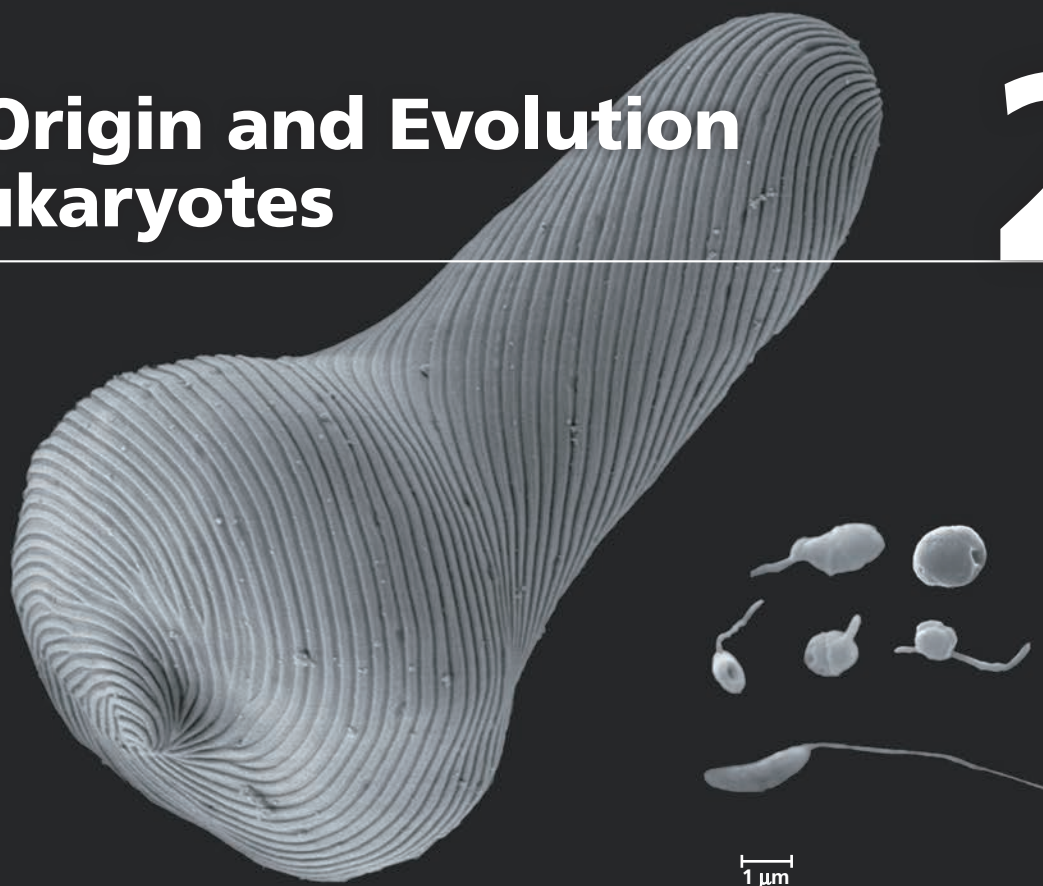
CONCEPT 27.3

Diverse nutritional and metabolic adaptations have evolved in prokaryotes (pp. 633–634)

- Nutritional diversity is much greater in prokaryotes than in eukaryotes and includes all four modes of nutrition: photoautotrophy, chemoautotrophy, photoheterotrophy, and chemoheterotrophy.

The Origin and Evolution of Eukaryotes

28



▲ **Figure 28.1** Which of these organisms are prokaryotes and which are eukaryotes?

KEY CONCEPTS

- 28.1** Most eukaryotes are single-celled organisms
- 28.2** Excavates include protists with modified mitochondria and protists with unique flagella
- 28.3** SAR is a highly diverse group of protists defined by DNA similarities
- 28.4** Red algae and green algae are the closest relatives of plants
- 28.5** Unikonts include protists that are closely related to fungi and animals
- 28.6** Protists play key roles in ecological communities

▼ **Trumpet-shaped protists (*Stentor coeruleus*)**

Living Small

Knowing that most prokaryotes are extremely small organisms, you might assume that **Figure 28.1** depicts six prokaryotes and one much larger eukaryote. But in fact, the only prokaryote is the organism immediately above the scale bar. The other six organisms are members of diverse, mostly unicellular groups of eukaryotes informally known as **protists**. These very small eukaryotes have intrigued biologists for more than 300 years, ever since the Dutch scientist Antoni van Leeuwenhoek first laid eyes on them under a light microscope. Some protists change their forms as they creep along using blob-like appendages, while others resemble tiny trumpets or miniature jewelry. Recalling his observations, van Leeuwenhoek wrote, “No more pleasant sight has met my eye than this, of so many thousands of living creatures in one small drop of water.”

The protists that fascinated van Leeuwenhoek continue to surprise us today. Metagenomic studies have revealed a treasure trove of previously unknown protists within the world of microscopic life. Many of these newly discovered organisms are just 0.5–2 μm in diameter—as small as many prokaryotes. Genetic and morphological studies have also shown that some protists are more closely related to plants, fungi, or animals than they are to other protists. As a result, the kingdom in which all protists once were classified, Protista, has been abandoned, and various protist lineages are now recognized as major groups in their own right. Most biologists still

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Get Ready for This Chapter

use the term *protist*, but only as a convenient way to refer to eukaryotes that are not plants, animals, or fungi.

In this chapter, you will become acquainted with some of the most significant groups of protists. You will learn about their structural and biochemical adaptations as well as their enormous impact on ecosystems, agriculture, industry, and human health.



HHMI Video: Seeing the Invisible: Van Leeuwenhoek's First Glimpses of the Microbial World



CONCEPT 28.1

Most eukaryotes are single-celled organisms

Protists, along with plants, animals, and fungi, are classified as eukaryotes; they are in domain Eukarya, one of the three domains of life. Unlike the cells of prokaryotes, eukaryotic cells have a nucleus and other membrane-enclosed organelles, such as mitochondria and the Golgi apparatus. Such organelles provide specific locations where particular cellular functions are accomplished, making the structure and organization of eukaryotic cells more complex than those of prokaryotic cells.

Eukaryotic cells also have a well-developed cytoskeleton that extends throughout the cell (see Figure 7.20). The cytoskeleton provides the structural support that enables eukaryotic cells to have asymmetric (irregular) forms, as well as to change in shape as they feed, move, or grow. In contrast, prokaryotic cells lack a well-developed cytoskeleton, thus limiting the extent to which they can maintain asymmetric forms or change shape over time.

We'll survey the diversity of eukaryotes throughout the rest of this unit, beginning in this chapter with the protists. As you explore this material, bear in mind that

- the organisms in most eukaryotic lineages are protists, and
- most protists are unicellular.

Thus, life differs greatly from how most of us commonly think of it. The large, multicellular organisms that we know best (plants, animals, and fungi) are the tips of just a few branches on the great tree of life (see Figure 22.21).

Structural and Functional Diversity in Protists

Given that they are classified in a number of different groups, it isn't surprising that few general characteristics of protists can be cited without exceptions. In fact, protists exhibit more structural and functional diversity than the eukaryotes with which we are most familiar—plants, animals, and fungi.

For example, most protists are unicellular, although there are some colonial and multicellular species. Single-celled protists are justifiably considered the simplest eukaryotes, but at the

cellular level, many protists are very complex—the most elaborate of all cells. In multicellular organisms, essential biological functions are carried out by organs. Unicellular protists carry out the same essential functions, but they do so using subcellular organelles, not multicellular organs. The organelles that protists use are mostly those discussed in Figure 7.8, including the nucleus, endoplasmic reticulum, Golgi apparatus, and lysosomes. Certain protists also rely on organelles not found in most other eukaryotic cells, such as contractile vacuoles that pump excess water from the protistan cell (see Figure 8.13).

Protists are also very diverse in their nutrition. Some protists are photoautotrophs and contain chloroplasts. Some are heterotrophs, absorbing organic molecules or ingesting larger food particles. Still other protists, called **mixotrophs**, combine photosynthesis and heterotrophic nutrition. Photoautotrophy, heterotrophy, and mixotrophy have all arisen independently in many different protist lineages.

Reproduction and life cycles also are highly varied among protists. Some protists are only known to reproduce asexually; others can also reproduce sexually or at least employ the sexual processes of meiosis and fertilization. All three basic types of sexual life cycles (see Figure 13.6) are represented among protists, along with some variations that do not quite fit any of these types. We will examine the life cycles of several protist groups later in this chapter.

Four Supergroups of Eukaryotes

Our understanding of the evolutionary history of eukaryotic diversity has been in flux in recent years. Not only has kingdom Protista been abandoned, but other hypotheses have been discarded as well. For example, many biologists once thought that the first lineage to have diverged from all other eukaryotes was the *amitochondriate protists*, organisms without conventional mitochondria and with fewer membrane-enclosed organelles than other protist groups. But recent structural and DNA data have undermined this hypothesis. Many of the so-called amitochondriate protists have been shown to have mitochondria—though reduced ones—and some of these organisms are now classified in distantly related groups.

The ongoing changes in our understanding of the phylogeny of protists pose challenges to students and instructors alike. Hypotheses about these relationships are a focus of scientific activity, changing rapidly as new data cause previous ideas to be modified or discarded. We'll focus here on one current hypothesis: the four supergroups of eukaryotes shown in **Figure 28.2**. Because the root of the eukaryotic tree is not known, all four supergroups are shown as diverging simultaneously from a common ancestor. We know that this is not correct, but we do not know which supergroup was the first to diverge from the others. In addition, while some of the groups in Figure 28.2 are well supported by morphological and DNA data, others are more controversial. As you read this chapter, it may be helpful to focus less on the specific names

of groups of organisms and more on why the organisms are important and how ongoing research is elucidating their evolutionary relationships.

Endosymbiosis in Eukaryotic Evolution

What gave rise to the enormous diversity of protists that exist today? There is abundant evidence that much of protistan diversity has its origins in **endosymbiosis**, a relationship between two species in which one organism lives inside the cell or cells of another organism (the host). In particular, as we discussed in Concept 25.3, structural, biochemical,

and DNA sequence data indicate that mitochondria and plastids are derived from prokaryotes that were engulfed by the ancestors of early eukaryotic cells. The evidence also suggests that mitochondria evolved before plastids. Thus, a defining moment in the origin of eukaryotes occurred when a host cell engulfed a bacterium that would later become an organelle found in all eukaryotes—the mitochondrion.

To determine which prokaryotic lineage gave rise to mitochondria, researchers have compared the DNA sequences of mitochondrial genes (mtDNA) to those found in major clades of bacteria and archaea. In the **Scientific Skills Exercise**, you will interpret one such set of DNA sequence comparisons.

SCIENTIFIC SKILLS EXERCISE

Interpreting Comparisons of Genetic Sequences

Which Prokaryotes Are Most Closely Related to Mitochondria? Early eukaryotes acquired mitochondria by endosymbiosis: A host cell engulfed an aerobic prokaryote that persisted within the cytoplasm to the mutual benefit of both cells. In studying which living prokaryotes might be most closely related to mitochondria, researchers compared ribosomal RNA (rRNA) sequences. Ribosomes perform critical cell functions. Hence, rRNA sequences are under strong selection and change slowly over time, making them suitable for comparing even distantly related species. In this exercise, you will interpret some of the research data to draw conclusions about the phylogeny of mitochondria.

How the Research Was Done Researchers isolated and cloned nucleotide sequences from the gene that codes for the small-subunit rRNA molecule for wheat (a eukaryote) and five bacterial species:

- Wheat, used as the source of mitochondrial rRNA genes
- Agrobacterium tumefaciens*, an alpha proteobacterium that lives within plant tissue and produces tumors in the host
- Comamonas testosteroni*, a beta proteobacterium
- Escherichia coli*, a well-studied gamma proteobacterium that inhabits human intestines
- Mycoplasma capricolum*, a gram-positive mycoplasma, which is the only group of bacteria lacking cell walls
- Anacystis nidulans*, a cyanobacterium

Data from the Research Cloned rRNA gene sequences for the six organisms were aligned and compared. The data table below, called a *comparison matrix*, summarizes the comparison of 617 nucleotide

Wheat, used as the source of mitochondrial RNA



positions from the gene sequences. Each value in the table is the percentage of the 617 nucleotide positions for which the pair of organisms have the same base. Any positions that were identical across the rRNA genes of all six organisms were omitted from this comparison matrix.

INTERPRET THE DATA

- First, make sure you understand how to read the comparison matrix. Find the cell that represents the comparison of *C. testosteroni* and *E. coli*. What value is given in this cell? What does that value signify about the comparable rRNA gene sequences in those two organisms? Explain why some cells have a dash rather than a value. Why are some cells shaded gray, with no value?
- Why did the researchers choose one plant mitochondrion and five bacterial species to include in the comparison matrix?
- Which bacterium has an rRNA gene that is most similar to that of the wheat mitochondrion? What is the significance of this similarity?

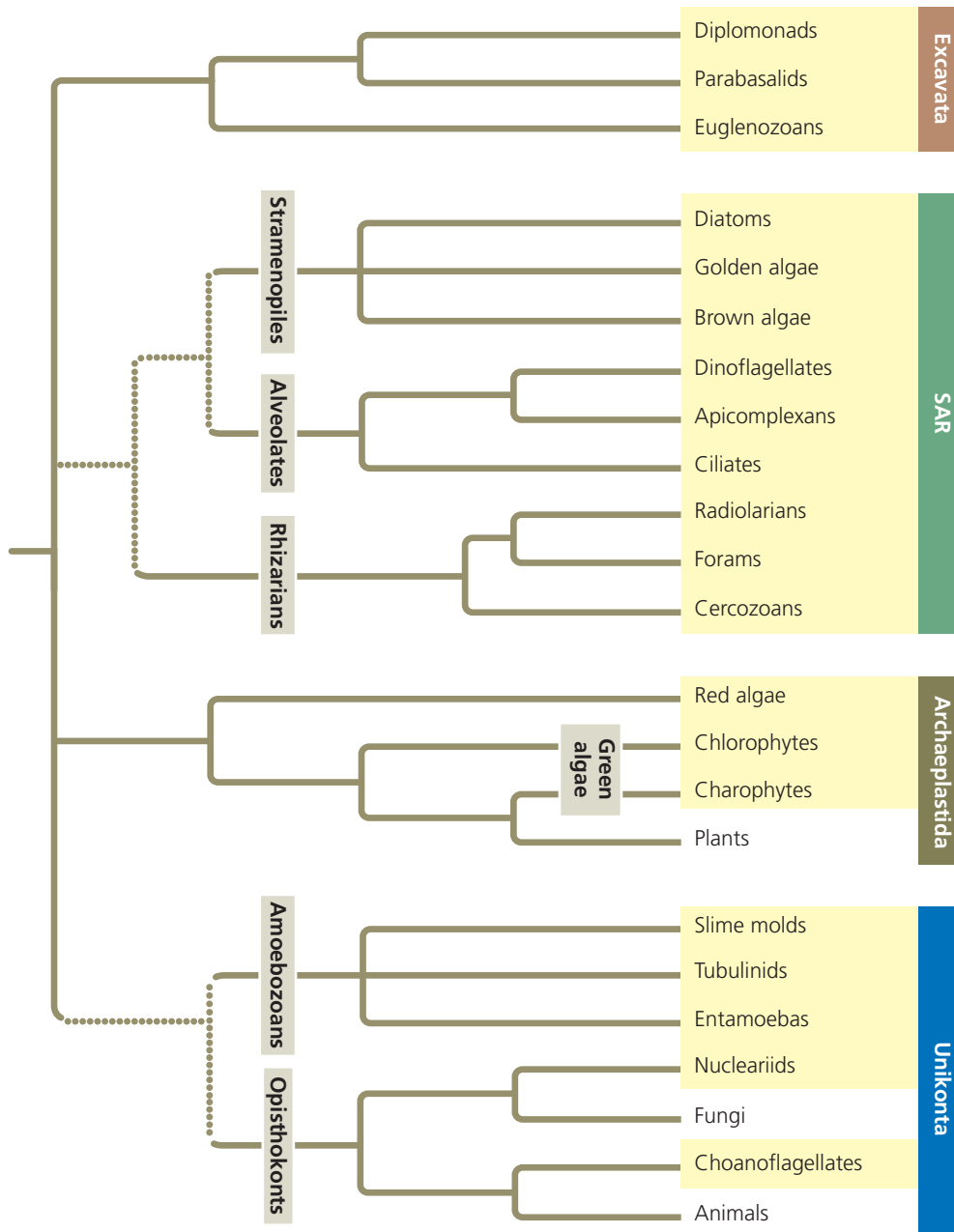
Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

	Wheat mitochondrion	<i>A. tumefaciens</i>	<i>C. testosteroni</i>	<i>E. coli</i>	<i>M. capricolum</i>	<i>A. nidulans</i>
Wheat mitochondrion	–	48	38	35	34	34
<i>A. tumefaciens</i>		–	55	57	52	53
<i>C. testosteroni</i>			–	61	52	52
<i>E. coli</i>				–	48	52
<i>M. capricolum</i>					–	50
<i>A. nidulans</i>						–

Data from D. Yang et al., Mitochondrial origins, *Proceedings of the National Academy of Sciences USA* 82:4443–4447 (1985).

▼ Figure 28.2 Exploring Protistan Diversity

The tree below represents a phylogenetic hypothesis for the relationships among eukaryotes on Earth today. The eukaryotic groups at the branch tips are related in larger “supergroups,” labeled vertically at the far right of the tree. Groups that were formerly classified in the kingdom Protista are highlighted in yellow. Dotted lines indicate evolutionary relationships that are uncertain and proposed clades that are under active debate. For clarity, this tree only includes representative clades from each supergroup. In addition, the recent discoveries of many new groups of eukaryotes indicate that eukaryotic diversity is actually much greater than shown here.



DRAW IT ► Draw a simplified version of this phylogenetic tree that shows only the four supergroups of eukaryotes. Now sketch how the tree would look if the unikonts were the sister group to all other eukaryotes.

■ Excavata

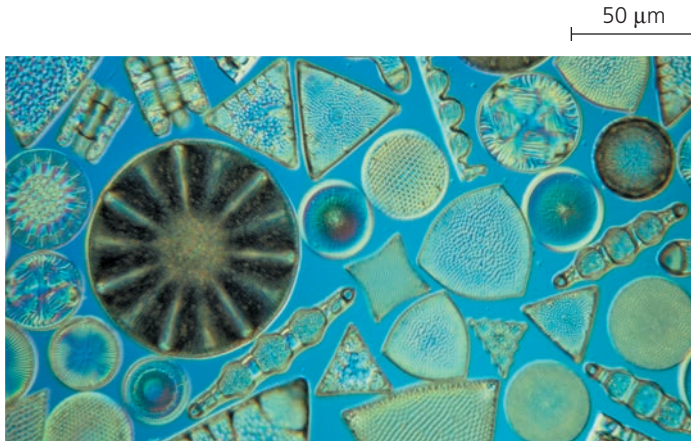
Some members of this supergroup have an “excavated” groove on one side of the cell body. Two major clades (the parabasalids and diplomonads) have highly reduced mitochondria; members of a third clade (the euglenozoans) have flagella that differ in structure from those of other organisms. Excavates include parasites such as *Giardia*, as well as many predatory and photosynthetic species.



***Giardia intestinalis*, a diplomonad parasite.** This diplomonad (colorized SEM), which lacks the characteristic surface groove of the Excavata, inhabits the intestines of mammals. It can infect people when they drink water contaminated with feces containing *Giardia* cysts. Drinking such water—even from a seemingly pristine stream—can cause severe diarrhea. Boiling the water kills the parasite.

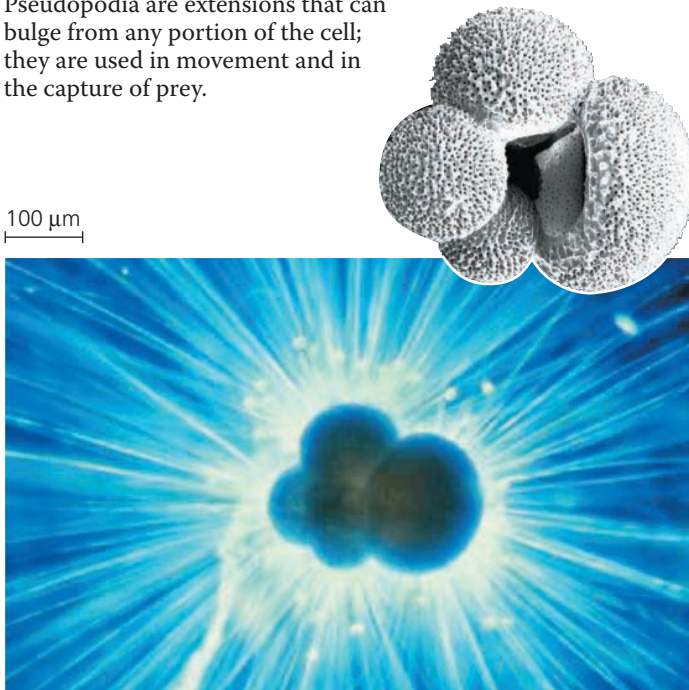
SAR

This supergroup contains (and is named after) three large and very diverse clades: Stramenopila, Alveolata, and Rhizaria. Stramenopiles include some of the most important photosynthetic organisms on Earth, such as the diatoms shown here. Alveolates also include photosynthetic species, as well as important pathogens, such as *Plasmodium*, which causes malaria. According to one current hypothesis, stramenopiles and alveolates originated by secondary endosymbiosis when a heterotrophic protist engulfed a red alga.



Diatom diversity. These beautiful single-celled protists are important photosynthetic organisms in aquatic communities (LM).

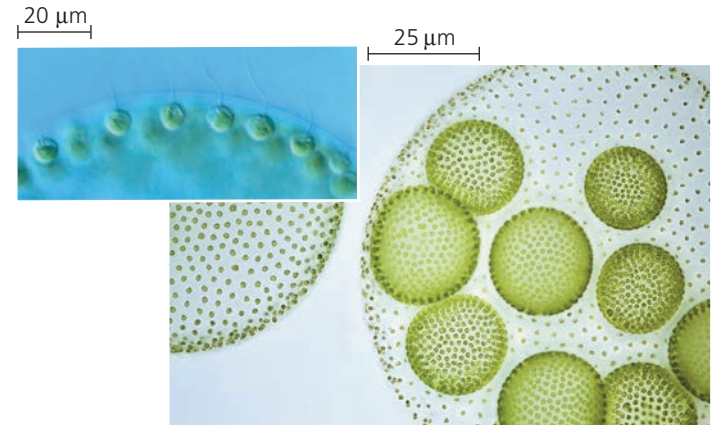
The rhizarian subgroup of SAR includes many species of amoebas, most of which have pseudopodia that are threadlike in shape. Pseudopodia are extensions that can bulge from any portion of the cell; they are used in movement and in the capture of prey.



Globigerina, a rhizarian in SAR. This species is a foram, a group whose members have threadlike pseudopodia that extend through pores in the shell, or test (LM). The inset shows a foram test, which is hardened by calcium carbonate.

Archaeplastida

This supergroup of eukaryotes includes red algae and green algae, along with plants. Red algae and green algae include unicellular species, colonial species, and multicellular species (including the green alga *Volvox*). Many of the large algae known informally as “seaweeds” are multicellular red or green algae. Protists in Archaeplastida include key photosynthetic species that form the base of the food web in many aquatic communities.



Volvox, a multicellular freshwater green alga. This alga has two types of differentiated cells, and so it is considered multicellular rather than colonial. It resembles a hollow ball whose wall is composed of hundreds of biflagellated cells (see inset LM) embedded in a gelatinous extracellular matrix; if isolated, these cells cannot reproduce. However, the alga also contains cells that are specialized for either sexual or asexual reproduction. The large algae shown here will eventually release the small “daughter” algae that can be seen within them (LM).

 **Video: Volvox**

Unikonta

This supergroup of eukaryotes includes amoebas that have lobe- or tube-shaped pseudopodia, as well as animals, fungi, and non-amoeba protists that are closely related to animals or fungi. According to one current hypothesis, the unikonts were the first eukaryotic supergroup to diverge from all other eukaryotes; however, this hypothesis has yet to be widely accepted.



A unikont amoeba. This amoeba, the tubulinid *Amoeba proteus*, is using its pseudopodia to move.

 **Video: Amoeba Pseudopodium**

Collectively, such studies indicate that mitochondria arose from an alpha proteobacterium (see Figure 27.16). Results from mtDNA sequence analyses also indicate that the mitochondria of protists, animals, fungi and plants descended from a single common ancestor, thus suggesting that mitochondria arose only once over the course of evolution. Similar analyses provide evidence that plastids descended from a single common ancestor—a cyanobacterium that was engulfed by a eukaryotic host cell.

Progress has also been made toward identifying the host cell that engulfed an alpha proteobacterium, thereby setting the stage for the origin of eukaryotes. In 2015, for example, researchers reported the discovery of a new group of archaea, the lokiarchaeotes. In phylogenomic analyses, this group was identified as the sister group of the eukaryotes and its genome was found to encode many eukaryote-specific features. Was the host cell that engulfed an alpha proteobacterium a lokiarchaeote? While this may have been the case, it is also possible that the host was closely related to the archaeans (but was not itself an archaean). Either way, current evidence indicates that the host was a relatively complex cell in which certain features of eukaryotic cells had evolved, such as a cytoskeleton that enabled it to change shape (and thereby engulf the alpha proteobacterium).

Plastid Evolution: A Closer Look

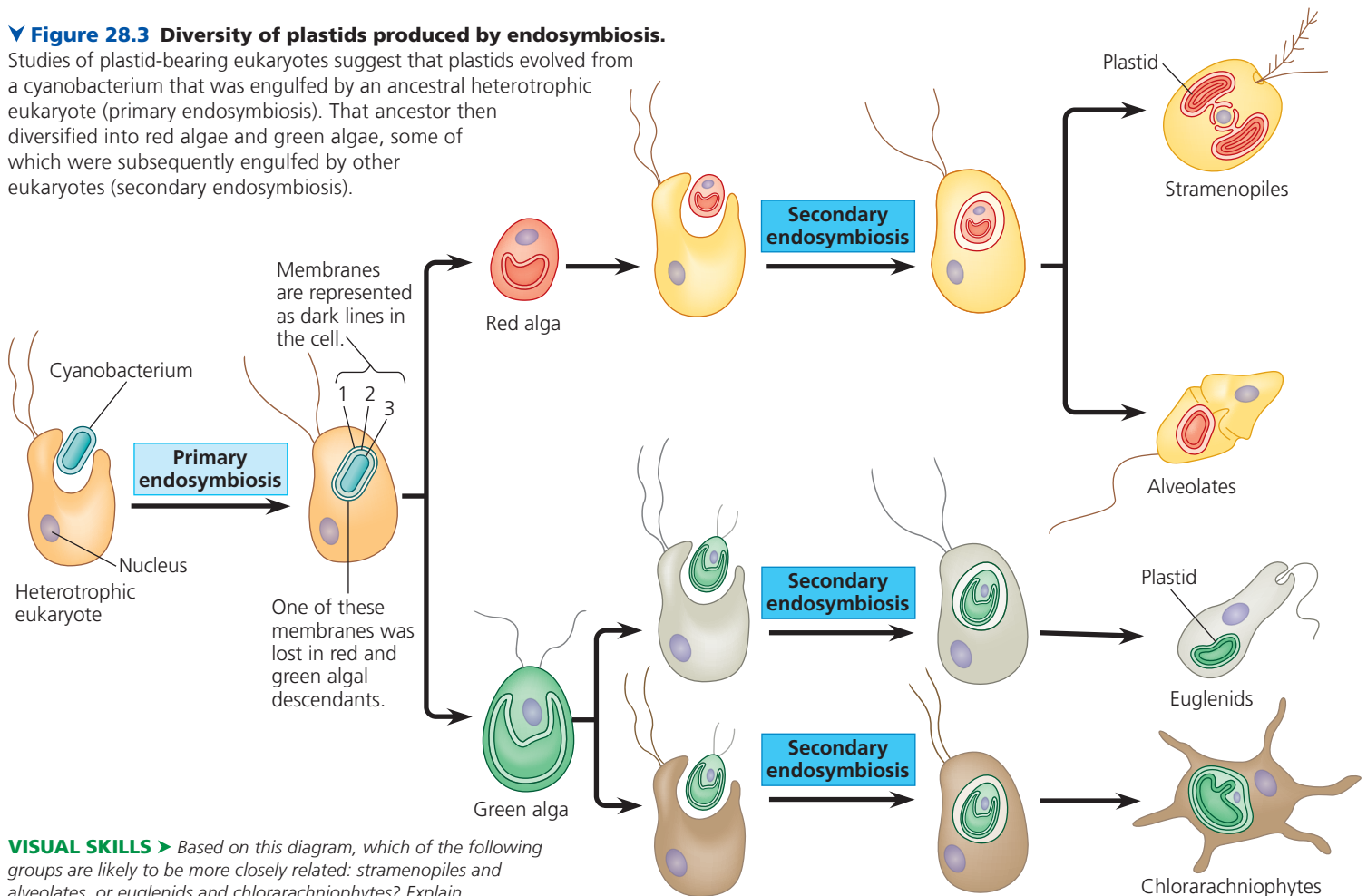
As you've seen, current evidence indicates that mitochondria are descended from a bacterium that was engulfed by a host cell that was an archaean (or a close relative of the archaeans). This event gave rise to the eukaryotes. There is also much evidence that later in eukaryotic history, a lineage of heterotrophic eukaryotes acquired an additional endosymbiont—a photosynthetic cyanobacterium—that then evolved into plastids. According to the hypothesis illustrated in **Figure 28.3**, this plastid-bearing lineage gave rise to two lineages of photosynthetic protists, or **algae**: red algae and green algae.

Let's examine some of the steps in Figure 28.3 more closely. First, recall that cyanobacteria are gram-negative and that gram-negative bacteria have two cell membranes, an inner plasma membrane and an outer membrane that is part of the cell wall (see Figure 27.3). Plastids in red algae and green algae are also surrounded by two membranes. Transport proteins in these membranes are homologous to proteins in the inner and outer membranes of cyanobacteria, providing further support for the hypothesis that plastids originated from a cyanobacterial endosymbiont.

On several occasions during eukaryotic evolution, red algae and green algae underwent **secondary endosymbiosis**,

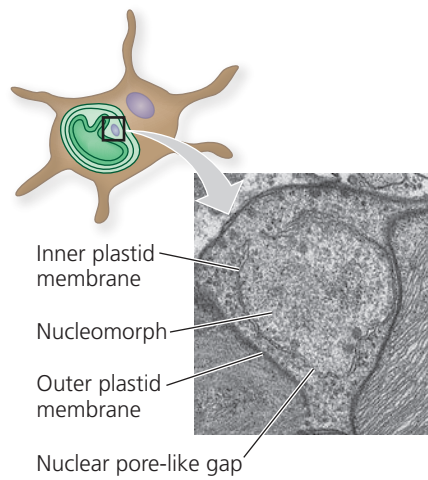
Figure 28.3 Diversity of plastids produced by endosymbiosis.

Studies of plastid-bearing eukaryotes suggest that plastids evolved from a cyanobacterium that was engulfed by an ancestral heterotrophic eukaryote (primary endosymbiosis). That ancestor then diversified into red algae and green algae, some of which were subsequently engulfed by other eukaryotes (secondary endosymbiosis).



VISUAL SKILLS ▶ Based on this diagram, which of the following groups are likely to be more closely related: stramenopiles and alveolates, or euglenids and chlorarachniophytes? Explain.

► **Figure 28.4**
Nucleomorph
within a plastid of a
chlorarachniophyte.



meaning they were ingested in the food vacuoles of heterotrophic eukaryotes and became endosymbionts themselves. For example, protists known as chlorarachniophytes likely evolved when a heterotrophic eukaryote engulfed a green alga. Evidence for this process can be found within the engulfed cell, which contains a tiny vestigial nucleus, called a *nucleomorph* (Figure 28.4). Genes from the nucleomorph are still transcribed, and their DNA sequences indicate that the engulfed cell was a green alga.

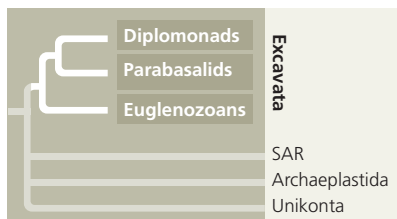
CONCEPT CHECK 28.1

1. Cite at least four examples of structural and functional diversity among protists.
2. Summarize the role of endosymbiosis in eukaryotic evolution.
3. **MAKE CONNECTIONS** ► After studying Figure 28.3, predict how many distinct genomes are contained within the cell of a chlorarachniophyte. Explain. (See Figures 7.17 and 7.18).

For suggested answers, see Appendix A.

CONCEPT 28.2

Excavates include protists with modified mitochondria and protists with unique flagella



Now that we have examined some of the broad patterns in eukaryotic evolution, we will look more closely at the four main groups of protists shown in Figure 28.2.

We begin with **Excavata** (the excavates), a clade that was originally proposed based on morphological studies of the

cytoskeleton. Some members of this diverse group also have an “excavated” feeding groove on one side of the cell body. The excavates include the diplomonads, parabasalids, and euglenozoans. Molecular data indicate that each of these three groups is monophyletic, and recent genomic studies support the monophyly of the excavate supergroup.

Diplomonads and Parabasalids

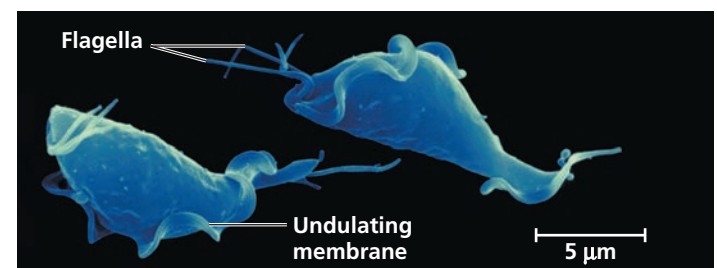
The protists in these two groups lack plastids and have highly reduced mitochondria (until recently, they were thought to lack mitochondria altogether). Most diplomonads and parabasalids are found in anaerobic environments.

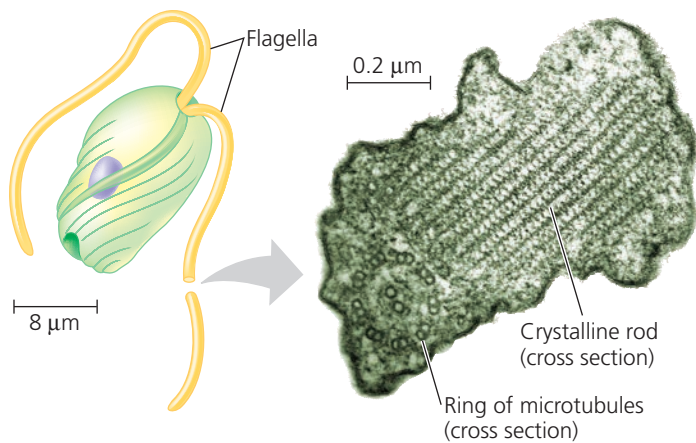
Diplomonads have reduced mitochondria called *mitosomes*. These organelles lack functional electron transport chains and hence cannot use oxygen to help extract energy from carbohydrates and other organic molecules. Instead, diplomonads get the energy they need from anaerobic biochemical pathways. Many diplomonads are parasites, including the infamous *Giardia intestinalis* (see Figure 28.2), which inhabits the intestines of mammals.

Structurally, diplomonads have two equal-sized nuclei and multiple flagella. Recall that eukaryotic flagella are extensions of the cytoplasm, consisting of bundles of microtubules covered by the cell’s plasma membrane (see Figure 7.24). They are quite different from prokaryotic flagella, which are filaments composed of globular proteins attached to the cell surface (see Figure 27.7).

Parabasalids also have reduced mitochondria; called *hydrogenosomes*, these organelles generate some energy anaerobically, releasing hydrogen gas as a by-product. The best-known parabasalid is *Trichomonas vaginalis*, a sexually transmitted parasite that infects some 5 million people each year. *T. vaginalis* travels along the mucus-coated lining of the human reproductive and urinary tracts by moving its flagella and by undulating part of its plasma membrane (Figure 28.5). In females, if the vagina’s normal acidity is disturbed, *T. vaginalis* can outcompete beneficial microorganisms there and infect the vagina. (*Trichomonas* infections also can occur in the urethra of males, though often without symptoms.) *T. vaginalis* has a gene that allows it to feed on the vaginal lining, promoting infection. Studies suggest that the protist acquired this gene by horizontal gene transfer from bacterial parasites in the vagina.

▼ **Figure 28.5** The parabasalid parasite *Trichomonas vaginalis* (colorized SEM).





▲ Figure 28.6 Euglenozoan flagellum. Most euglenozoans have a crystalline rod inside one of their flagella (the TEM is a flagellum shown in cross section). The rod lies alongside the 9 + 2 ring of microtubules found in all eukaryotic flagella (compare with Figure 7.24).

Euglenozoans

Protists called **euglenozoans** belong to a diverse clade that includes predatory heterotrophs, photosynthetic autotrophs, mixotrophs, and parasites. The main morphological feature that distinguishes protists in this clade is the presence of a rod with either a spiral or a crystalline structure inside each of their flagella (**Figure 28.6**). The two best-studied groups of euglenozoans are the kinetoplastids and the euglenids.

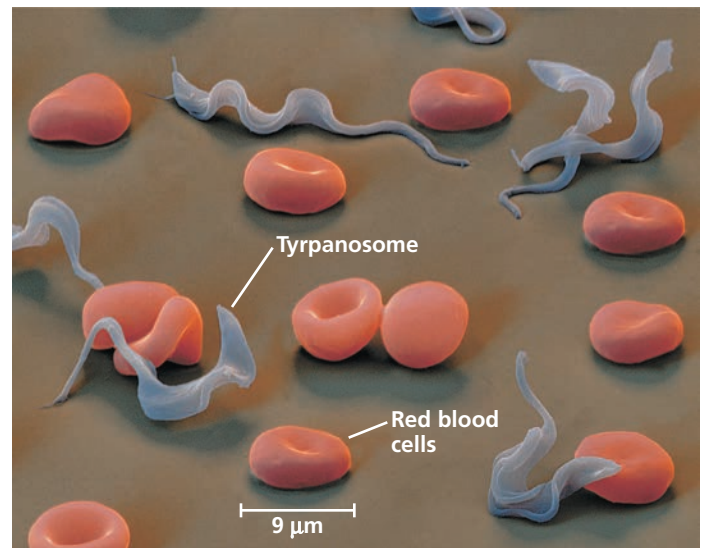
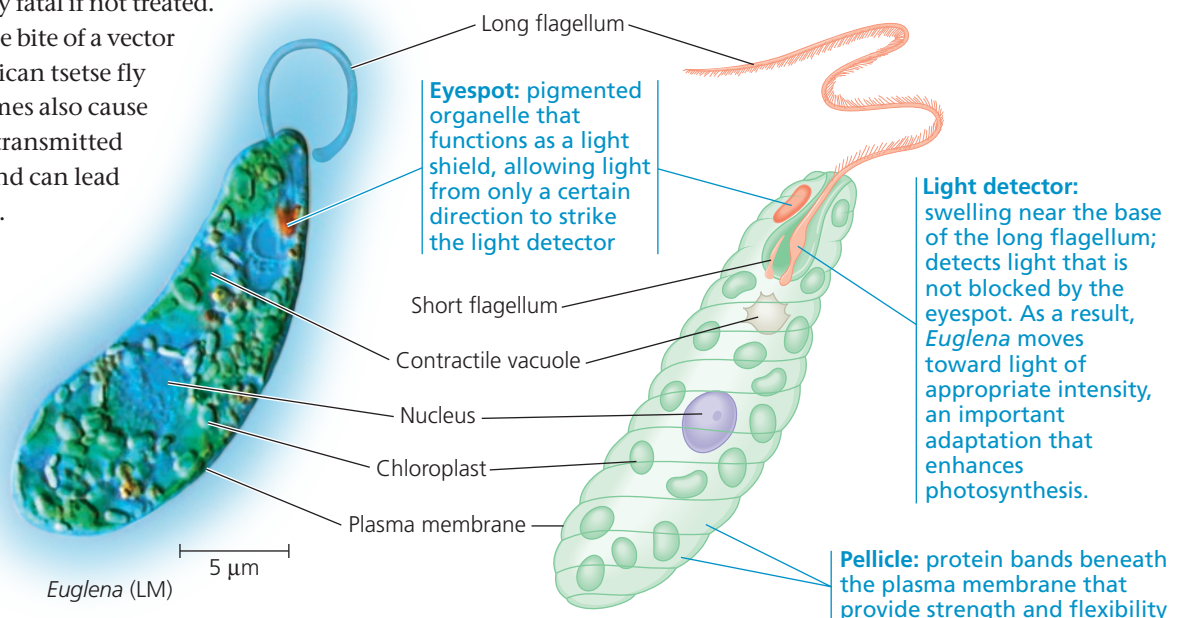
Kinetoplastids

Protists called **kinetoplastids** have a single, large mitochondrion that contains an organized mass of DNA called a *kinetoplast*. These protists include species that feed on prokaryotes in freshwater, marine, and moist terrestrial ecosystems, as well as species that parasitize animals, plants, and other protists. For example, kinetoplastids in the genus *Trypanosoma* infect humans and cause sleeping sickness, a neurological disease that is invariably fatal if not treated.

The infection occurs via the bite of a vector (carrier) organism, the African tsetse fly (**Figure 28.7**). Trypanosomes also cause Chagas' disease, which is transmitted by bloodsucking insects and can lead to congestive heart failure.

► Figure 28.8
***Euglena*, a euglenid commonly found in pond water.**

Video: *Euglena*



▲ Figure 28.7 *Trypanosoma*, the kinetoplastid that causes sleeping sickness (colorized SEM).

Trypanosomes evade immune responses with an effective “bait-and-switch” defense. The surface of a trypanosome is coated with millions of copies of a single protein. However, before the host’s immune system can recognize the protein and mount an attack, new generations of the parasite switch to another surface protein with a different molecular structure. Frequent changes in the surface protein prevent the host from developing immunity. (The Scientific Skills Exercise in Chapter 47 explores this topic further.) About a third of *Trypanosoma*’s genome is dedicated to producing these surface proteins.

Euglenids

A **euglenid** has a pocket at one end of the cell from which one or two flagella emerge (**Figure 28.8**). Some euglenids are

mixotrophs: They perform photosynthesis when sunlight is available, but when it is not, they can become heterotrophic, absorbing organic nutrients from their environment. Many other euglenids engulf prey by phagocytosis.

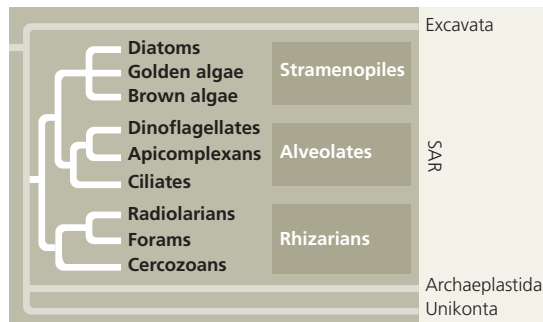
CONCEPT CHECK 28.2

1. Why is it difficult for humans to develop immunity against trypanosomes?
2. **WHAT IF? >** DNA sequence data for a diplomonad, a euglenid, a plant, and an unidentified protist suggest that the unidentified species is most closely related to the diplomonad. Further studies reveal that the unknown species has fully functional mitochondria. Based on these data, at what point on the phylogenetic tree in Figure 28.2 did the mystery protist's lineage probably diverge from other eukaryote lineages? Explain.

For suggested answers, see Appendix A.

CONCEPT 28.3

SAR is a highly diverse group of protists defined by DNA similarities



Our second supergroup, referred to as **SAR**, was proposed recently based on whole-genome DNA sequence analyses. These studies have found that three major clades of protists—the stramenopiles, alveolates, and rhizarians—form a monophyletic supergroup. This supergroup contains a large, extremely diverse collection of protists. To date, this supergroup has not received a formal name but is instead known by the first letters of its major clades: SAR.

Some morphological and DNA sequence data suggest that two of these groups, the stramenopiles and alveolates, originated more than a billion years ago, when a common ancestor of these two clades engulfed a single-celled, photosynthetic red alga. Because red algae are thought to have originated by primary endosymbiosis (see Figure 28.3), such an origin for the stramenopiles and alveolates is referred to as secondary endosymbiosis. Others question this idea, noting that some species in these groups lack plastids or their remnants (including any trace of plastid genes in their nuclear DNA).

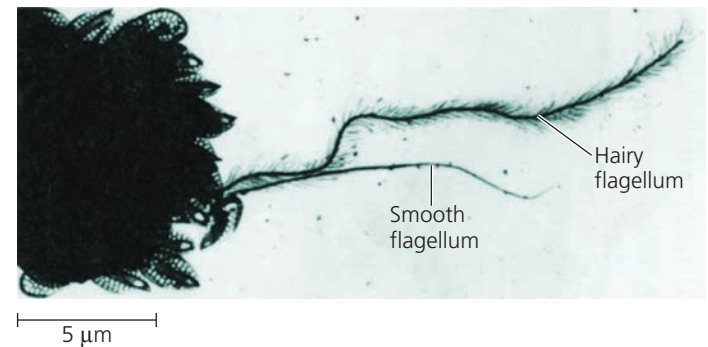
As its lack of a formal name suggests, SAR is one of the most controversial of the four supergroups we describe in this chapter. Even so, for many scientists, this supergroup

represents the best current hypothesis for the phylogeny of the three large protist clades to which we now turn.

Stramenopiles

One major subgroup of SAR, the **stramenopiles**, includes some of the most important photosynthetic organisms on the planet. Their name (from the Latin *stramen*, straw, and *pilos*, hair) refers to their characteristic flagellum, which has numerous fine, hairlike projections. In most stramenopiles, this “hairy” flagellum is paired with a shorter “smooth” (nonhairy) flagellum (**Figure 28.9**). Here we’ll focus on three groups of stramenopiles: diatoms, golden algae, and brown algae.

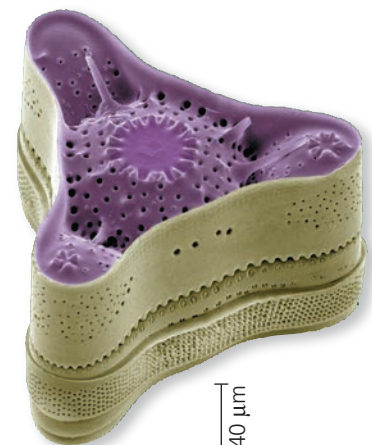
▼ **Figure 28.9 Stramenopile flagella.** Most stramenopiles, such as *Synura petersenii*, have two flagella: one covered with fine, stiff hairs and a shorter one that is smooth.



Diatoms

A key group of photosynthetic protists, **diatoms** are unicellular algae that have a unique glass-like wall made of silicon dioxide embedded in an organic matrix (**Figure 28.10**). The wall consists of two parts that overlap like a shoe box and its lid. These walls provide effective protection from the crushing jaws of predators: Live diatoms can withstand pressures as great as 1.4 million kg/m², equal to the pressure under each leg of a table supporting an elephant!

With an estimated 100,000 living species, diatoms are a highly diverse group of protists (see Figure 28.2). They are among the most abundant photosynthetic organisms both in the ocean and in lakes: One bucket of water scooped from the surface of the sea may contain millions of these microscopic algae. The abundance of diatoms in the past is also evident in



► **Figure 28.10** The diatom *Triceratium morlandii* (colorized SEM).

the fossil record, where massive accumulations of fossilized diatom walls are major constituents of sediments known as *diatomaceous earth*. These sediments are mined for their quality as a filtering medium and for many other uses.

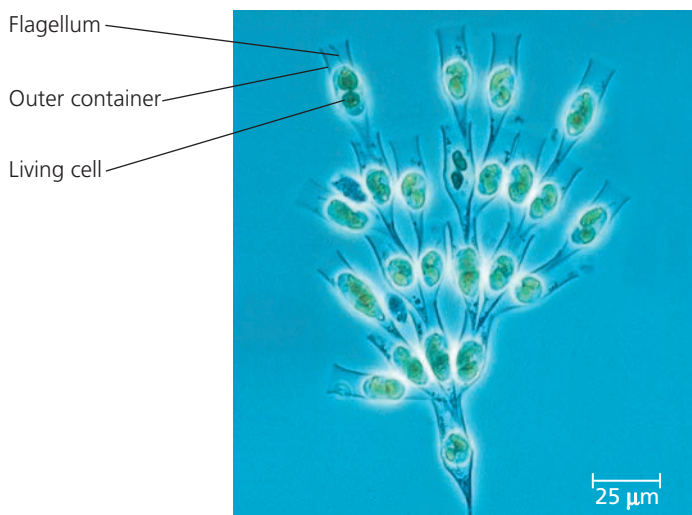
Diatoms are so widespread and abundant that their photosynthetic activity affects global carbon dioxide (CO₂) levels. Diatoms have this effect in part because of events that occur during episodes of rapid population growth, or *blooms*, when ample nutrients are available. Typically, diatoms are eaten by a variety of protists and invertebrates, but during a bloom, many escape this fate. When these uneaten diatoms die, their bodies sink to the ocean floor. It takes decades, or even centuries, for diatoms that sink to the ocean floor to be broken down by bacteria and other decomposers. As a result, the carbon in their bodies remains there for some time, rather than being released immediately as CO₂ as the decomposers respire. The overall effect of these events is that CO₂ absorbed by diatoms during photosynthesis is transported, or “pumped,” to the ocean floor.

With an eye toward reducing global warming by lowering atmospheric CO₂ levels, some scientists advocate promoting diatom blooms by fertilizing the ocean with essential nutrients such as iron. In a 2012 study, researchers found that CO₂ was indeed pumped to the ocean floor after iron was added to a small region of the ocean. Further tests are planned to examine whether iron fertilization has undesirable side effects (such as oxygen depletion or the production of nitrous oxide, a more potent greenhouse gas than CO₂).

Golden Algae

The characteristic color of **golden algae** results from their yellow and brown carotenoids. The cells of golden algae are typically biflagellated, with both flagella attached near one end of the cell. Most species are unicellular, but some are colonial (**Figure 28.11**).

▼ **Figure 28.11** *Dinobryon*, a colonial golden alga found in fresh water (LM).



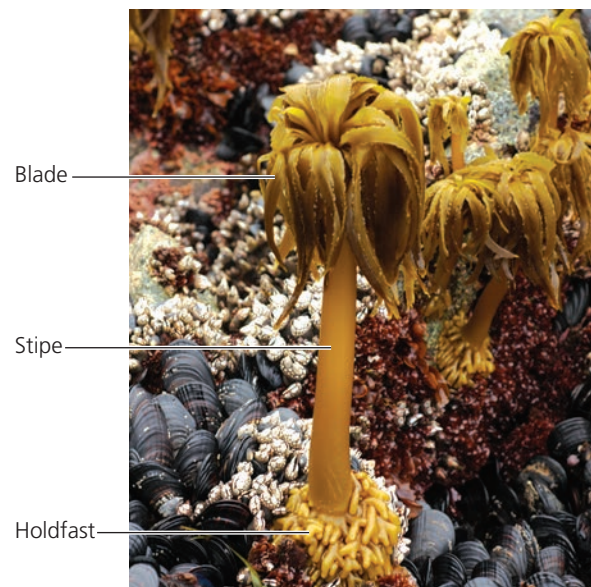
Many golden algae are components of freshwater and marine *plankton*, communities of mostly microscopic organisms that drift in currents near the water's surface. While all golden algae are photosynthetic, some species are mixotrophic. These mixotrophs can absorb dissolved organic compounds or ingest food particles, including living cells, by phagocytosis. If environmental conditions deteriorate, many species form protective cysts that can survive for decades.

Brown Algae

The largest and most complex algae are **brown algae**. All are multicellular, and most are marine. Brown algae are especially common along temperate coasts that have cold-water currents. They owe their characteristic brown or olive color to the carotenoids in their plastids.

Many of the species commonly called “seaweeds” are brown algae. Some brown algal seaweeds have specialized structures that resemble organs in plants, such as a rootlike **holdfast**, which anchors the alga, and a stemlike **stipe**, which supports the leaflike **blades** (**Figure 28.12**). Unlike plants, however, brown algae lack true tissues and organs. Moreover, morphological and DNA data show that these similarities evolved independently in the algal and plant lineages and are thus analogous, not homologous. In addition, while plants have adaptations (such as rigid stems) that provide support against gravity, brown algae have adaptations that enable their main photosynthetic surfaces (the leaf-like blades) to be near the water surface. Some brown algae accomplish this task with gas-filled, bubble-shaped floats.

▼ **Figure 28.12** Seaweeds: adapted to life at the ocean's margins. The sea palm (*Postelsia*) lives on rocks along the coast of the northwestern United States and western Canada. The body of this brown alga is well adapted to maintaining a firm foothold despite the crashing surf.



Giant brown algae known as kelps that live in deep waters have such floats in their blades, which are attached to stipes that can rise as much as 60 m from the seafloor—more than half the length of a football field.

Brown algae are important commodities for humans. Some species are eaten, such as *Laminaria* (Japanese “kombu”), which is used in soups. In addition, the cell walls of brown algae contain a gel-forming substance, called algin, which is used to thicken many processed foods, including pudding and salad dressing.

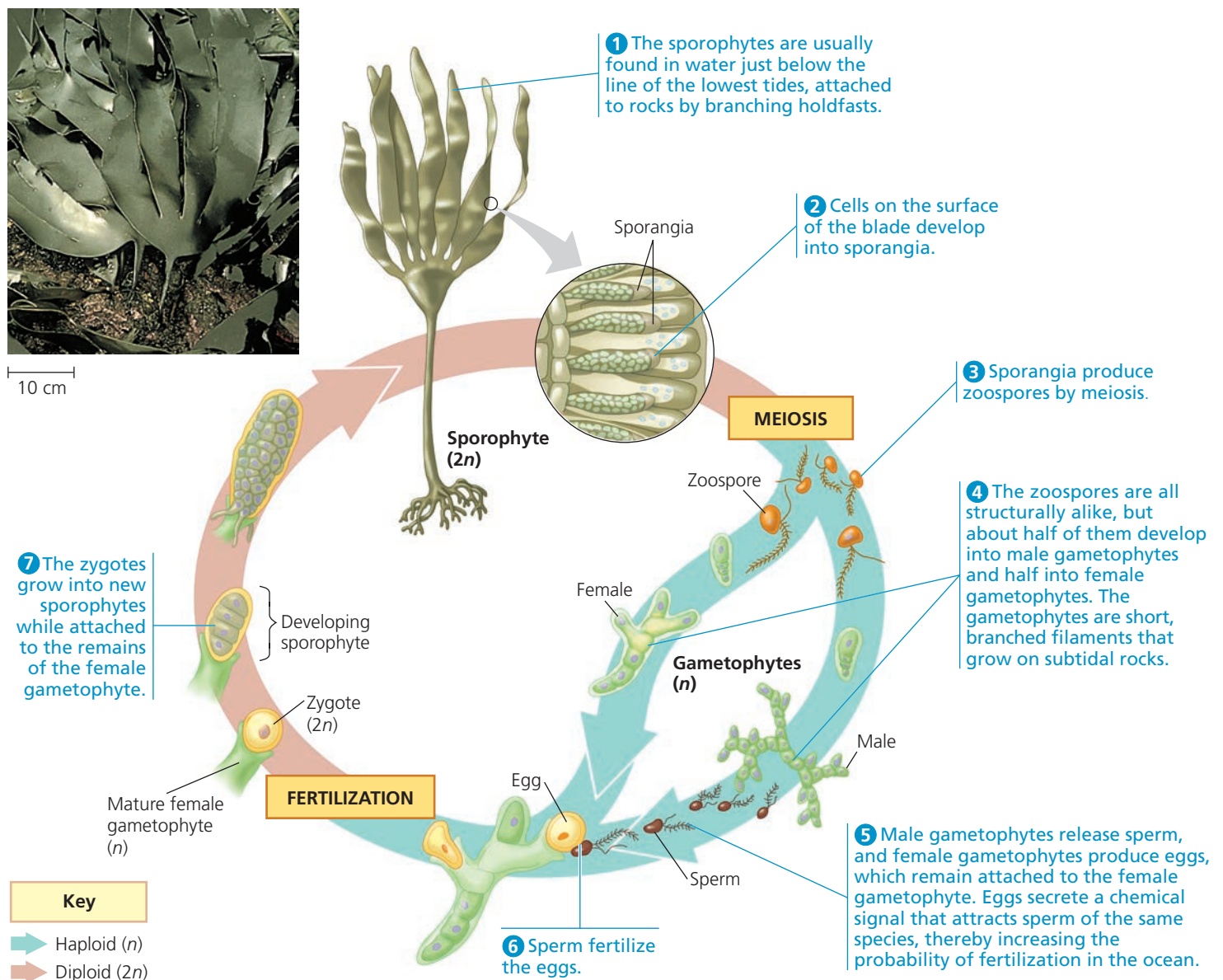
Alternation of Generations

A variety of life cycles have evolved among the multicellular algae. The most complex life cycles include an **alternation**

of generations, the alternation of multicellular haploid and diploid forms. Although haploid and diploid conditions alternate in *all* sexual life cycles—human gametes, for example, are haploid—the term *alternation of generations* applies only to life cycles in which both haploid and diploid stages are multicellular. As you will read in Concept 29.1, alternation of generations also evolved in plants.

The complex life cycle of the brown alga *Laminaria* provides an example of alternation of generations (**Figure 28.13**). The diploid individual is called the *sporophyte* because it produces spores. The spores are haploid and move by means of flagella; they are called zoospores. The zoospores develop into haploid, multicellular male and female *gametophytes*, which produce gametes. The union of two gametes (fertilization)

▼ **Figure 28.13** The life cycle of the brown alga *Laminaria*: an example of alternation of generations.



VISUAL SKILLS ► Based on this diagram, are the sperm shown in **5** genetically identical to one another? Explain.

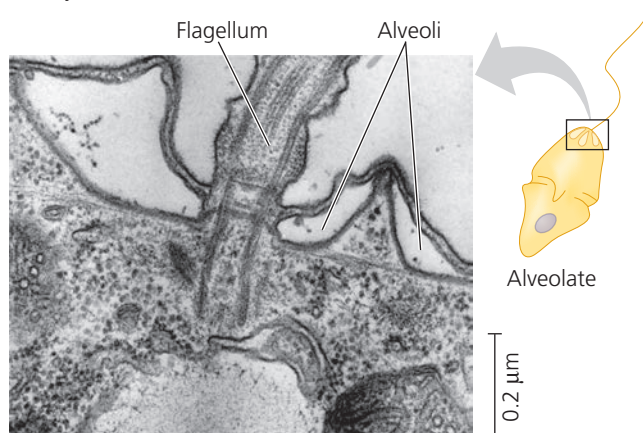
results in a diploid zygote, which matures and gives rise to a new multicellular sporophyte.

In *Laminaria*, the two generations are **heteromorphic**, meaning that the sporophytes and gametophytes are structurally different. Other algal life cycles have an alternation of **isomorphic** generations, in which the sporophytes and gametophytes look similar to each other, although they differ in chromosome number.

Alveolates

Members of the next subgroup of SAR, the **alveolates**, have membrane-enclosed sacs (alveoli) just under the plasma membrane (**Figure 28.14**). Alveolates are abundant in many habitats and include a wide range of photosynthetic and heterotrophic protists. We'll discuss three alveolate clades here: a group of flagellates (the dinoflagellates), a group of parasites (the apicomplexans), and a group of protists that move using cilia (the ciliates).

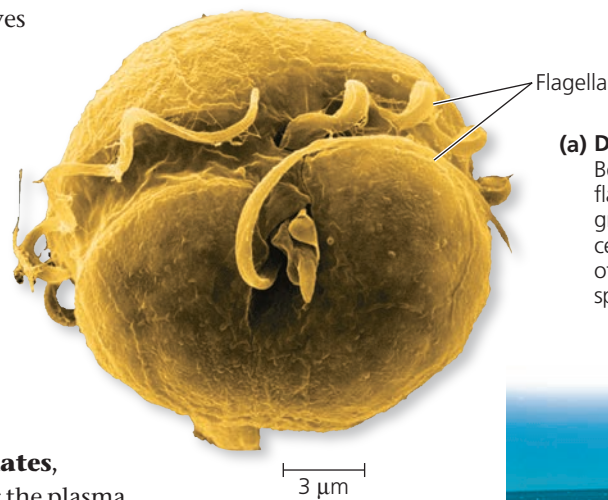
▼ **Figure 28.14 Alveoli.** These sacs under the plasma membrane are a characteristic that distinguishes alveolates from other eukaryotes (TEM).



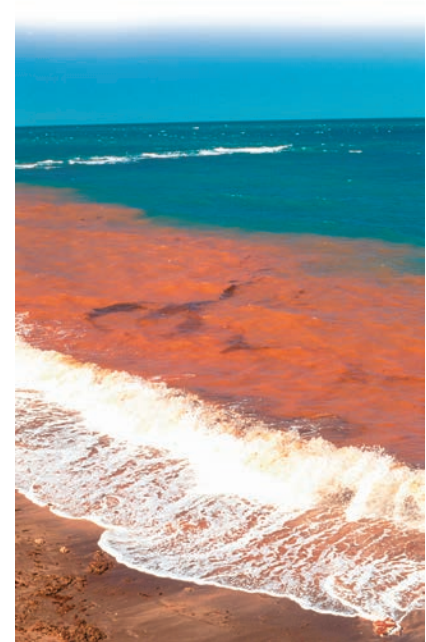
Dinoflagellates

The cells of many **dinoflagellates** are reinforced by cellulose plates. Two flagella located in grooves in this “armor” make dinoflagellates (from the Greek *dinos*, whirling) spin as they move through the waters of their marine and freshwater communities (**Figure 28.15a**). Although their ancestors may have originated by secondary endosymbiosis (see Figure 28.3), roughly half of all dinoflagellates are now purely heterotrophic. Others are important species of *phytoplankton* (photosynthetic plankton, which include photosynthetic bacteria as well as algae); many photosynthetic dinoflagellates are mixotrophic.

Periods of explosive population growth (blooms) in dinoflagellates sometimes cause a phenomenon called “red tide”



(a) **Dinoflagellate flagella.** Beating of the spiral flagellum, which lies in a groove that encircles the cell, makes this specimen of *Pfiesteria shumwayae* spin (colorized SEM).



(b) **Red tide in the Gulf of Carpentaria in northern Australia.** The red color is due to high concentrations of a carotenoid-containing dinoflagellate.

▲ **Figure 28.15 Dinoflagellates.**

Video: Dinoflagellate

(**Figure 28.15b**). The blooms make coastal waters appear brownish red or pink because of the presence of carotenoids, the most common pigments in dinoflagellate plastids. Toxins produced by certain dinoflagellates have caused massive kills of invertebrates and fishes. Humans who eat molluscs that have accumulated the toxins are affected as well, sometimes fatally.

Apicomplexans

Nearly all **apicomplexans** are parasites of animals—and virtually all animal species examined so far are attacked by these parasites. The parasites spread through their host as tiny infectious cells called *sporozoites*. Apicomplexans are so named because one end (the *apex*) of the sporozoite cell contains a *complex* of organelles specialized for penetrating host cells and tissues. Although apicomplexans are not photosynthetic, recent data show that they retain a modified plastid (apicoplast), most likely of red algal origin.

▼ **Figure 28.16** The two-host life cycle of *Plasmodium*, the apicomplexan that causes malaria.

? Are morphological differences between sporozoites, merozoites, and gametocytes caused by different genomes or by differences in gene expression? Explain.

Animation: Life Cycle of a Malaria Parasite

8 An oocyst develops from the zygote in the wall of the mosquito's gut. The oocyst releases thousands of sporozoites, which migrate to the mosquito's salivary gland.

7 Fertilization occurs in the mosquito's digestive tract, and a zygote forms.

6 Gametes form from gametocytes; each male gametocyte produces several slender male gametes.

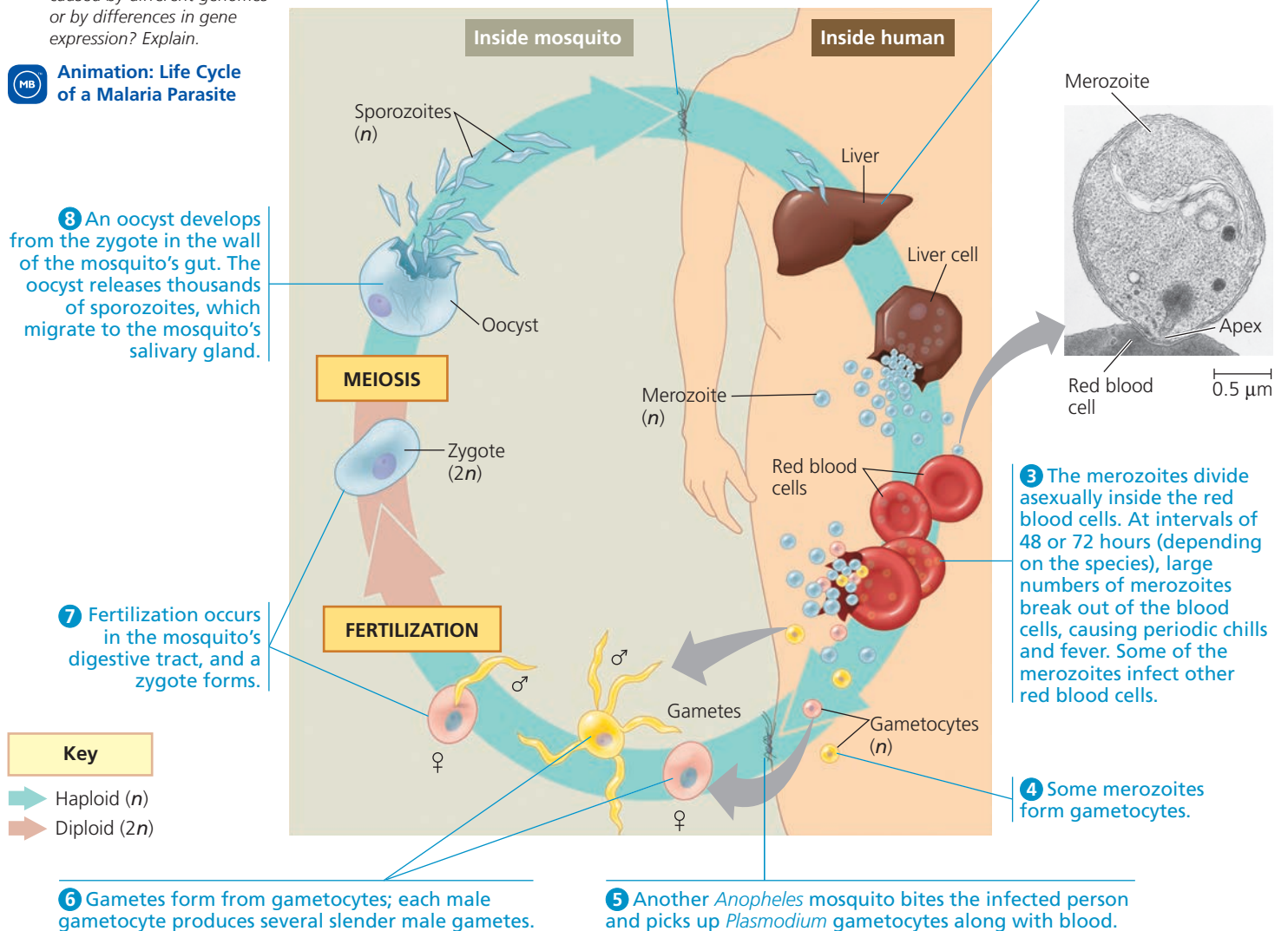
1 An infected *Anopheles* mosquito bites a person, injecting *Plasmodium* sporozoites in its saliva.

2 The sporozoites enter the person's liver cells. After several days, the sporozoites undergo multiple divisions and become merozoites, which use their apical complex to penetrate red blood cells (see TEM below).

3 The merozoites divide asexually inside the red blood cells. At intervals of 48 or 72 hours (depending on the species), large numbers of merozoites break out of the blood cells, causing periodic chills and fever. Some of the merozoites infect other red blood cells.

4 Some merozoites form gametocytes.

5 Another *Anopheles* mosquito bites the infected person and picks up *Plasmodium* gametocytes along with blood.



Most apicomplexans have intricate life cycles with both sexual and asexual stages. Those life cycles often require two or more host species for completion. For example, *Plasmodium*, the parasite that causes malaria, lives in both mosquitoes and humans (**Figure 28.16**).

Historically, malaria has rivaled tuberculosis as the leading cause of human death by infectious disease. The incidence of malaria was diminished in the 1960s by insecticides that reduced carrier populations of *Anopheles* mosquitoes and by drugs that killed *Plasmodium* in humans. But the emergence of resistant varieties of both *Anopheles* and *Plasmodium* has led to a resurgence of malaria. About 200 million people in the tropics are currently infected, and 600,000 die each year. In regions where malaria is common, the lethal effects of this disease have resulted in the evolution of high frequencies of

the sickle-cell allele; for an explanation of this connection, see Figure 23.18.

The search for malarial vaccines has been hampered by the fact that *Plasmodium* lives mainly inside cells, hidden from the host's immune system. And, like trypanosomes, *Plasmodium* continually changes its surface proteins. Even so, significant progress was made in 2015, when European regulators approved the world's first licensed malarial vaccine. However, this vaccine, which targets a protein on the surface of sporozoites, provides only partial protection against malaria. As a result, researchers continue to study other potential vaccine targets, including the apicoplast. This approach may be effective because the apicoplast is a modified plastid; as such, it descended from a cyanobacterium and hence has different metabolic pathways from those in the human patients.

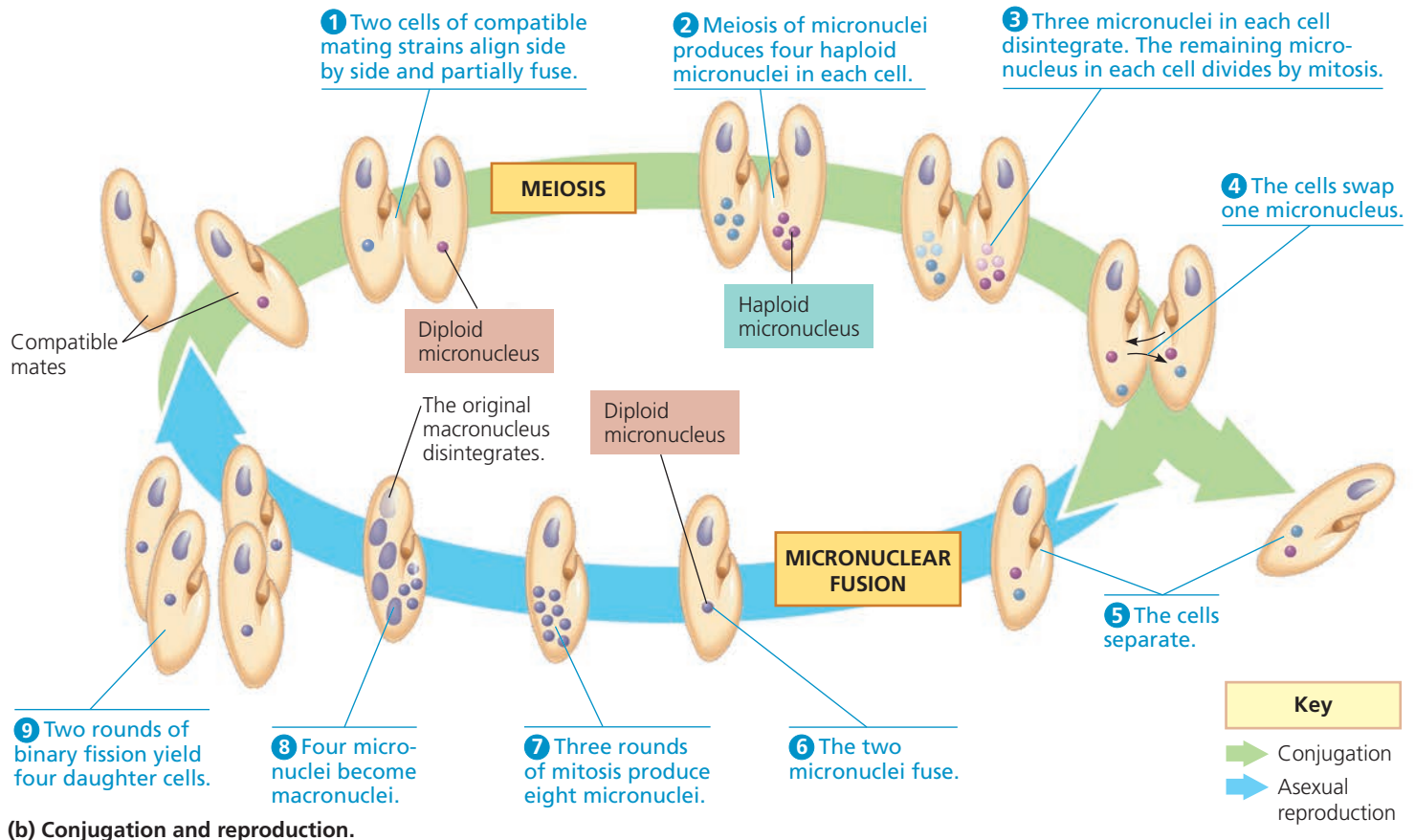
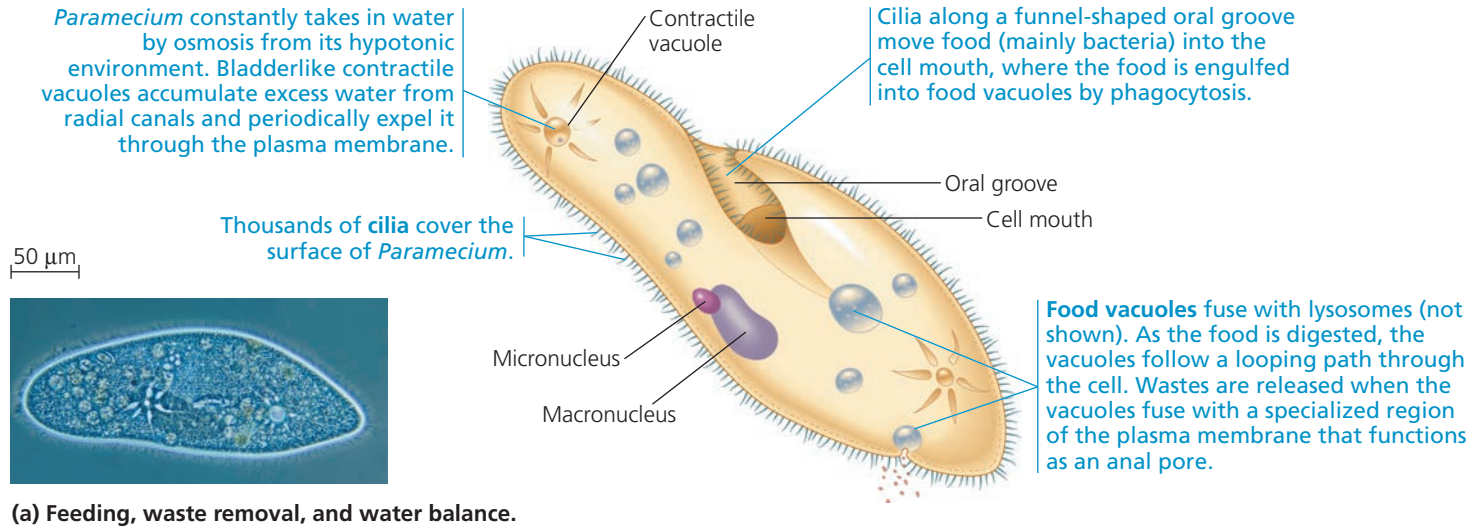
Ciliates

The **ciliates** are a large and varied group of protists named for their use of cilia to move and feed (**Figure 28.17a**). Most ciliates are predators, typically of bacteria or of other protists. Their cilia may completely cover the cell surface or may be clustered in a few rows or tufts. In certain species, rows

of tightly packed cilia function collectively in locomotion. Other ciliates scurry about on leg-like structures constructed from many cilia bonded together.

A distinctive feature of ciliates is the presence of two types of nuclei: tiny micronuclei and large macronuclei. A cell has one or more nuclei of each type. Genetic variation

▼ **Figure 28.17** Structure and function in the ciliate *Paramecium caudatum*.



MAKE CONNECTIONS ► The events shown in steps 5 and 6 of this diagram have a similar overall effect to what event in the human life cycle (see Figure 13.5)? Explain.

Video: Paramecium

results from **conjugation**, a sexual process in which two individuals exchange haploid micronuclei but do not reproduce (**Figure 28.17b**). Ciliates generally reproduce asexually by binary fission, during which the existing macronucleus disintegrates and a new one is formed from the cell's micronuclei. Each macronucleus typically contains multiple copies of the ciliate's genome. Genes in the macronucleus control the everyday functions of the cell, such as feeding, waste removal, and maintaining water balance.



Video: Ciliate Movement in *Stentor*

Rhizarians

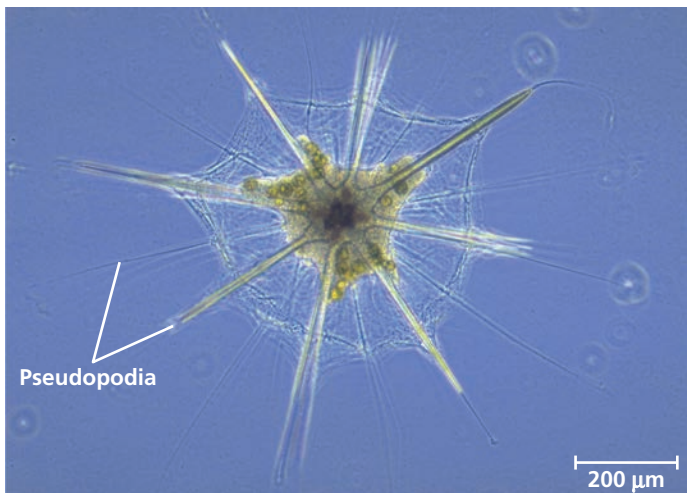
Our next subgroup of SAR is the **rhizarians**. Many species in this group are **amoebas**, protists that move and feed by means of **pseudopodia**, extensions that may bulge from almost anywhere on the cell surface. As it moves, an amoeba extends a pseudopodium and anchors the tip; more cytoplasm then streams into the pseudopodium. Amoebas do not constitute a monophyletic group; instead, they are dispersed across many distantly related eukaryotic taxa. Most amoebas that are rhizarians differ morphologically from other amoebas by having threadlike pseudopodia. Rhizarians also include flagellated (non-amoeboid) protists that feed using threadlike pseudopodia.

We'll examine three groups of rhizarians here: radiolarians, forams, and cercozoans.

Radiolarians

The protists called **radiolarians** have delicate, intricately symmetrical internal skeletons that are generally made of silica. The pseudopodia of these mostly marine protists radiate from the central body (**Figure 28.18**) and are reinforced by bundles of microtubules. The microtubules are covered by a thin layer of cytoplasm, which engulfs smaller microorganisms that become attached to the pseudopodia. Cytoplasmic

▼ **Figure 28.18** A radiolarian. Numerous threadlike pseudopodia radiate from the central body of this radiolarian (LM).



streaming then carries the captured prey into the main part of the cell. After radiolarians die, their skeletons settle to the seafloor, where they have accumulated as an ooze that is hundreds of meters thick in some locations.

Forams

The protists called **foraminiferans** (from the Latin *foramen*, little hole, and *ferre*, to bear), or **forams**, are named for their porous shells, called **tests** (see Figure 28.2). Foram tests consist of a single piece of organic material that typically is hardened with calcium carbonate. The pseudopodia that extend through the pores function in swimming, test formation, and feeding. Many forams also derive nourishment from the photosynthesis of symbiotic algae that live within the tests.

Forams are found in both the ocean and fresh water. Most species live in sand or attach themselves to rocks or algae, but some live as plankton. The largest forams, though single-celled, have tests several centimeters in diameter.

Ninety percent of all identified species of forams are known from fossils. Along with the calcium-containing remains of other protists, the fossilized tests of forams are part of marine sediments, including sedimentary rocks that are now land formations. Foram fossils are excellent markers for correlating the ages of sedimentary rocks in different parts of the world. Researchers are also studying these fossils to obtain information about climate change and its effects on the oceans and their life (**Figure 28.19**).

Cercozoans

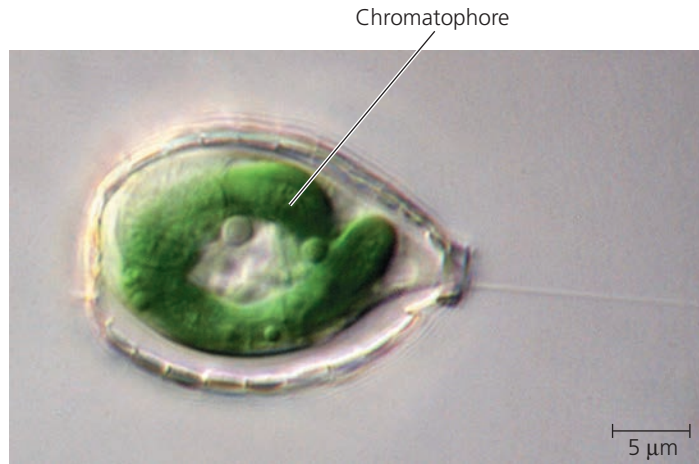
First identified in molecular phylogenies, the **cercozoans** are a large group of amoeboid and flagellated protists that feed using threadlike pseudopodia. Cercozoan protists are common inhabitants of marine, freshwater, and soil ecosystems.

▼ **Figure 28.19** Fossil forams. By measuring the magnesium content in fossilized forams like these, researchers seek to learn how ocean temperatures have changed over time. Forams take up more magnesium in warmer water than in colder water.



▼ Figure 28.20 A second case of primary endosymbiosis?

The cercozoan *Paulinella* conducts photosynthesis in a unique sausage-shaped structure called a chromatophore (LM). Chromatophores are surrounded by a membrane with a peptidoglycan layer, suggesting that they are derived from a bacterium. DNA evidence indicates that chromatophores are derived from a different cyanobacterium than that from which plastids are derived.



Most cercozoans are heterotrophs. Many are parasites of plants, animals, or other protists; many others are predators. The predators include the most important consumers of bacteria in aquatic and soil ecosystems, along with species that eat other protists, fungi, and even small animals. One small group of cercozoans, the chlorarachniophytes (mentioned earlier in the discussion of secondary endosymbiosis), are mixotrophic: These organisms ingest smaller protists and bacteria as well as perform photosynthesis. At least one other cercozoan, *Paulinella chromatophora*, is an autotroph, deriving its energy from light and its carbon from CO₂. As described in Figure 28.20, *Paulinella* appears to represent an intriguing additional evolutionary example of a eukaryotic lineage that obtained its photosynthetic apparatus directly from a cyanobacterium.

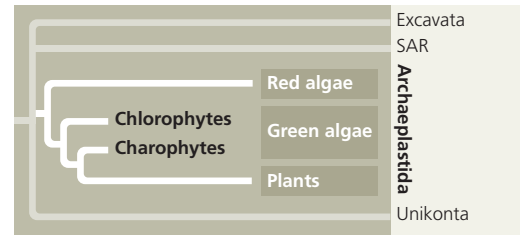
CONCEPT CHECK 28.3

1. Why is it difficult to develop a vaccine against malaria?
2. **WHAT IF? >** Would you expect the plastid DNA of photosynthetic dinoflagellates, diatoms, and golden algae to be more similar to the nuclear DNA of plants (domain Eukarya) or to the chromosomal DNA of cyanobacteria (domain Bacteria)? Explain.
3. **MAKE CONNECTIONS >** Which of the three life cycles in Figure 13.6 exhibits alternation of generations? How does it differ from the other two?
4. **MAKE CONNECTIONS >** Review Figures 10.2 and 11.6, and then summarize how CO₂ and O₂ are both used and produced by chlorarachniophytes and other aerobic algae.

For suggested answers, see Appendix A.

CONCEPT 28.4

Red algae and green algae are the closest relatives of plants



As described earlier, morphological and molecular evidence indicates that plastids arose when a heterotrophic protist acquired a cyanobacterial endosymbiont. Later, photosynthetic descendants of this ancient protist evolved into red algae and green algae (see Figure 28.3), and the lineage that produced green algae then gave rise to plants. Together, red algae, green algae, and plants make up our third eukaryotic supergroup, which is called **Archaeplastida**. Archaeplastida is a monophyletic group that descended from the ancient protist that engulfed a cyanobacterium. We will examine plants in Chapters 29 and 30; here we will look at the diversity of their closest algal relatives, red algae and green algae.

Red Algae

Many of the 6,000 known species of **red algae** (rhodophytes, from the Greek *rhodos*, red) are reddish, owing to the photosynthetic pigment phycoerythrin, which masks the green of chlorophyll (Figure 28.21). However, other species (those adapted to shallow water) have less phycoerythrin. As a result, red algal species may be greenish red in very shallow water, bright red at moderate depths, and almost black in deep water. Some species lack pigmentation altogether and live as heterotrophic parasites on other red algae.

Red algae are abundant in the warm coastal waters of tropical oceans. Some of their photosynthetic pigments, including phycoerythrin, allow them to absorb blue and green light, which penetrate relatively far into the water. A species of red alga has been discovered near the Bahamas at a depth of more than 260 m. There are also a small number of freshwater and terrestrial species.

Most red algae are multicellular. Although none are as big as the giant brown kelps, the largest multicellular red algae are included in the informal designation “seaweeds.” You may have eaten one of these multicellular red algae, *Porphyra* (Japanese “nori”), as crispy sheets or as a wrap for sushi (see Figure 28.21). Red algae reproduce sexually and have diverse life cycles in which alternation of generations is common. However, unlike other algae, red algae do not have flagellated gametes, so they depend on water currents to bring gametes together for fertilization.

▼ **Figure 28.21 Red algae.**

► ***Bonnemaisonia hamifera*.** This red alga has a filamentous form.

20 cm



8 mm

◄ **Dulse (*Palmaria palmata*).** This edible species has a "leafy" form.

▼ **Nori.** The red alga *Porphyra* is the source of a traditional Japanese food.



The seaweed is grown on nets in shallow coastal waters.



Paper-thin, glossy sheets of dried nori make a mineral-rich wrap for rice, seafood, and vegetables in sushi.

Green Algae

The grass-green chloroplasts of **green algae** have a structure and pigment composition much like the chloroplasts of plants. Molecular systematics and cellular morphology leave little doubt that green algae and plants are closely related. In fact, some systematists now advocate including green algae in an expanded "plant" kingdom, Viridiplantae (from the Latin *viridis*, green). Phylogenetically, this change makes sense, since otherwise the green algae are a paraphyletic group.

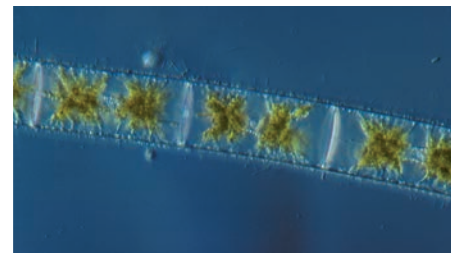
Green algae can be divided into two main groups, the charophytes and the chlorophytes. The charophytes include the algae most closely related to plants, and we will discuss them along with plants in Chapter 29.

The second group, the chlorophytes (from the Greek *chloros*, green), includes more than 7,000 species. Most live in fresh water, but there are also many marine and some terrestrial species. The simplest chlorophytes are unicellular organisms such as *Chlamydomonas*, which resemble gametes of more complex chlorophytes. Various species of unicellular chlorophytes live independently in aquatic habitats as phytoplankton or inhabit damp soil. Some live symbiotically within other eukaryotes, contributing part of their photosynthetic output to the food supply of their hosts. Still other chlorophytes live in environments exposed to intense visible and ultraviolet radiation; these species are protected by radiation-blocking compounds in their cytoplasm, cell wall, or zygote coat.

Larger size and greater complexity evolved in green algae by three different mechanisms:

1. The formation of colonies of individual cells, as seen in *Zygnema* (**Figure 28.22a**) and other species whose filamentous forms contribute to the stringy masses known as pond scum.
2. The formation of true multicellular bodies by cell division and differentiation, as in *Volvox* (see Figure 28.2) and *Ulva* (**Figure 28.22b**).
3. The repeated division of nuclei with no cytoplasmic division, as in *Caulerpa* (**Figure 28.22c**).

▼ **Figure 28.22 Examples of large chlorophytes.**



(a) ***Zygnema*, a common pond alga.** This filamentous charophyte features two star-shaped chloroplasts in each cell.

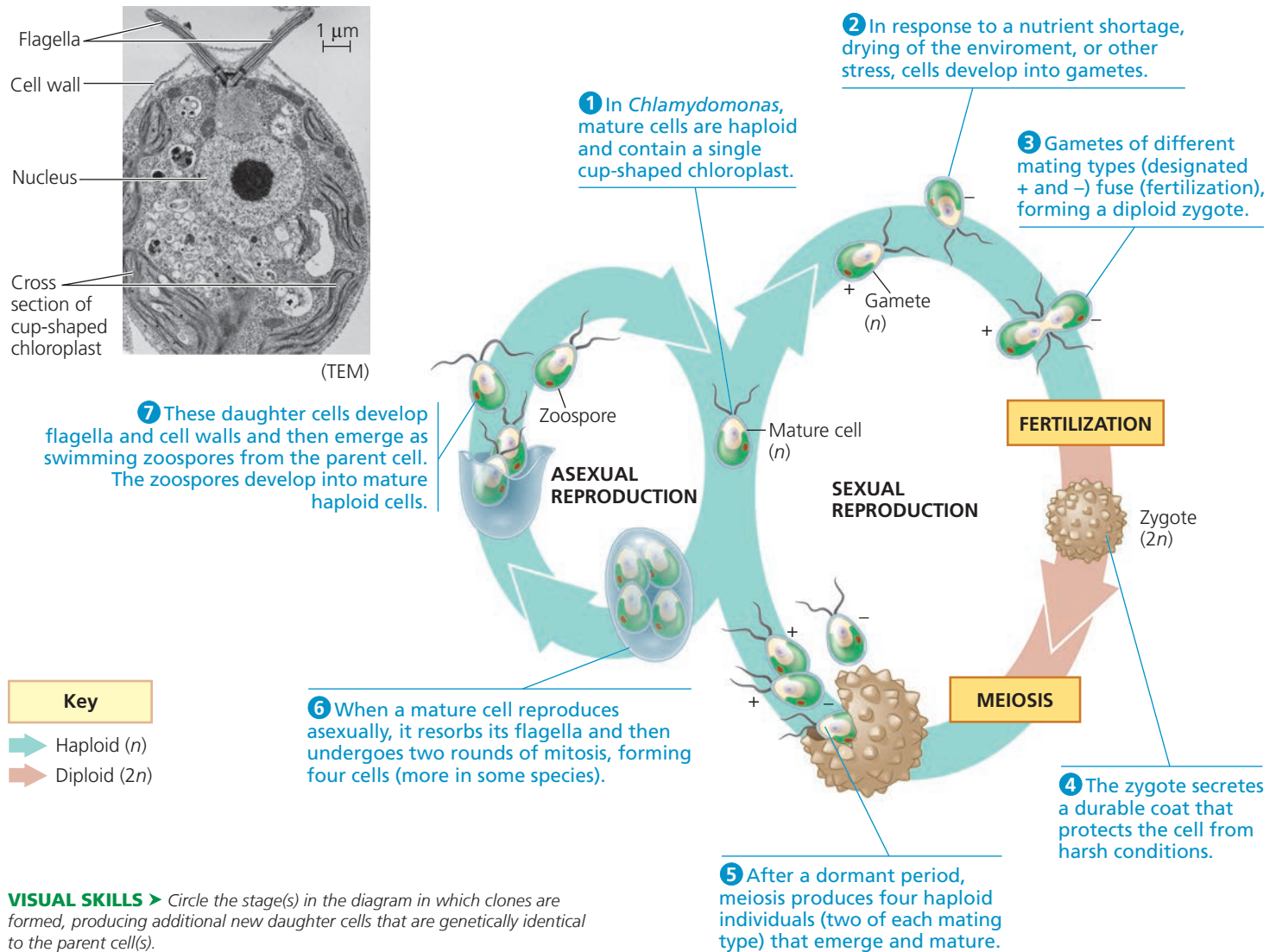


(b) ***Ulva*, or sea lettuce.** This multicellular, edible chlorophyte has differentiated structures, such as its leaflike blades and a rootlike holdfast that anchors the alga.

(c) ***Caulerpa*, an intertidal chlorophyte.** The branched filaments lack cross-walls and thus are multinucleate. In effect, the body of this alga is one huge "supercell."



▼ **Figure 28.23** The life cycle of *Chlamydomonas*, a unicellular chlorophyte.



VISUAL SKILLS ➤ Circle the stage(s) in the diagram in which clones are formed, producing additional new daughter cells that are genetically identical to the parent cell(s).

Most chlorophytes have complex life cycles, with both sexual and asexual reproductive stages. Nearly all species of chlorophytes reproduce sexually by means of biflagellated gametes that have cup-shaped chloroplasts (Figure 28.23). Alternation of generations has evolved in some chlorophytes, including *Ulva*.

Animation: Alternation of Generations in a Protist

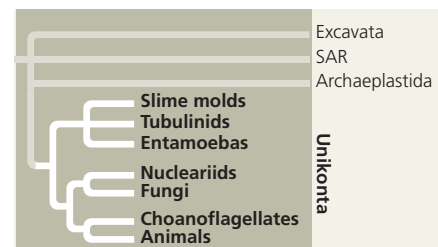
CONCEPT CHECK 28.4

1. Contrast red algae and brown algae.
2. Why is it accurate to say that *Ulva* is truly multicellular but *Caulerpa* is not?
3. **WHAT IF?** ➤ Suggest a possible reason why species in the green algal lineage may have been more likely to colonize land than species in the red algal lineage.

For suggested answers, see Appendix A.

CONCEPT 28.5

Unikonta include protists that are closely related to fungi and animals



Unikonta is an extremely diverse supergroup of eukaryotes that includes animals, fungi, and some protists. There are two major clades of unikonta, the amoebozoans and the

opisthokonts (animals, fungi, and closely related protist groups). Each of these two major clades is strongly supported by molecular systematics. The close relationship between amoebozoans and opisthokonts is more controversial. Support for this close relationship is provided by comparisons of myosin proteins and by some (but not all) studies based on multiple genes or whole genomes.

Another controversy involving the unikonts concerns the root of the eukaryotic tree. Recall that the root of a phylogenetic tree anchors the tree in time: Branch points close to the root are the oldest. At present, the root of the eukaryotic tree is uncertain; hence, we do not know which supergroup of eukaryotes was the first to diverge from all other eukaryotes. Some hypotheses, such as the amitochondriate hypothesis described earlier, have been abandoned, but researchers have yet to agree on an alternative. If the root of the eukaryotic tree were known, it would help scientists infer characteristics of the common ancestor of all eukaryotes.

In trying to determine the root of the eukaryotic tree, researchers have based their phylogenies on different sets of genes, some of which have produced conflicting results. Researchers have also tried a different approach, based on tracing the occurrence of a rare evolutionary event (**Figure 28.24**). Results from this “rare event” approach indicate that Excavata, SAR, and Archaeplastida share a more recent common ancestor than any of them does with Unikonta. This suggests that the root of the tree is located between the unikonts and all other eukaryotes, which implies that the unikonts were the first eukaryotic supergroup to diverge from all other eukaryotes. This idea remains controversial and will require more supporting evidence to be widely accepted.

Amoebozoans

The **amoebozoan** clade includes many species of amoebas that have lobe- or tube-shaped pseudopodia rather than the threadlike pseudopodia found in rhizarians. Amoebozoans include slime molds, tubulinids, and entamoebas.

Slime Molds

Slime molds, or mycetozoans (from the Latin, meaning “fungus animals”), once were thought to be fungi because, like fungi, they produce fruiting bodies that aid in spore dispersal. However, DNA sequence analyses indicate that the resemblance between slime molds and fungi is a case of evolutionary convergence. DNA sequence analyses also show that slime molds descended from unicellular ancestors—an example of the independent origin of multicellularity in eukaryotes.

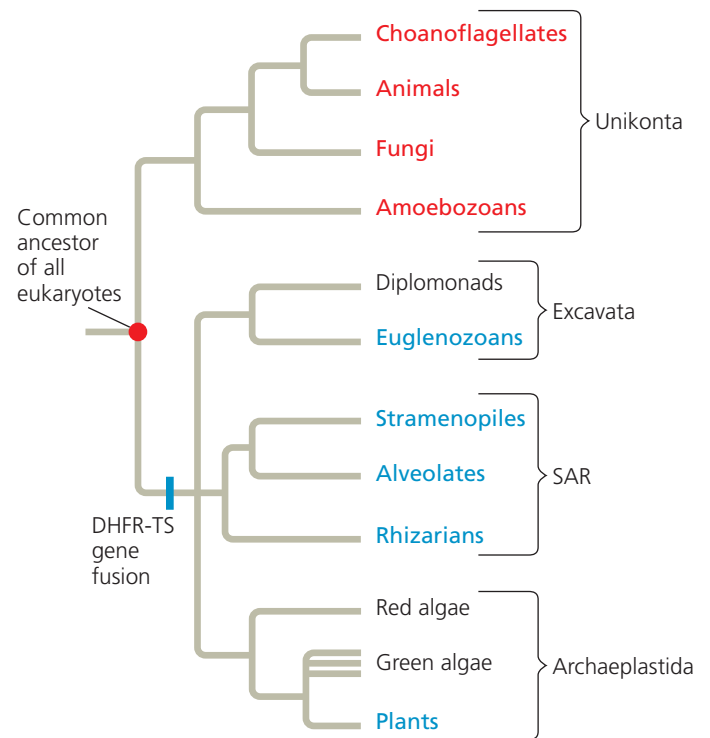
Slime molds have diverged into two main branches, plasmodial slime molds and cellular slime molds. We’ll compare their characteristics and life cycles.

▼ Figure 28.24

Inquiry What is the root of the eukaryotic tree?

Experiment Responding to the difficulty in determining the root of the eukaryotic phylogenetic tree, Alexandra Stechmann and Thomas Cavalier-Smith proposed a new approach. They studied two genes, one coding for the enzyme dihydrofolate reductase (DHFR) and the other coding for the enzyme thymidylate synthase (TS). The scientists’ approach took advantage of a rare evolutionary event: In some organisms, the genes for DHFR and TS have fused, leading to the production of a single protein with both enzyme activities. Stechmann and Cavalier-Smith amplified (using PCR; see Figure 19.8) and sequenced the genes for DHFR and TS in nine species (one choanoflagellate, two amoebozoans, one euglenozoan, one stramenopile, one alveolate, and three rhizarians). They combined their data with previously published data for species of bacteria, animals, plants, and fungi.

Results The bacteria studied all have separate genes coding for DHFR and TS, suggesting that this is the ancestral condition (red dot on the tree below). Other taxa with separate genes are denoted by red type. Fused genes are a derived character, found in certain members (blue type) of the supergroups Excavata, SAR, and Archaeplastida:

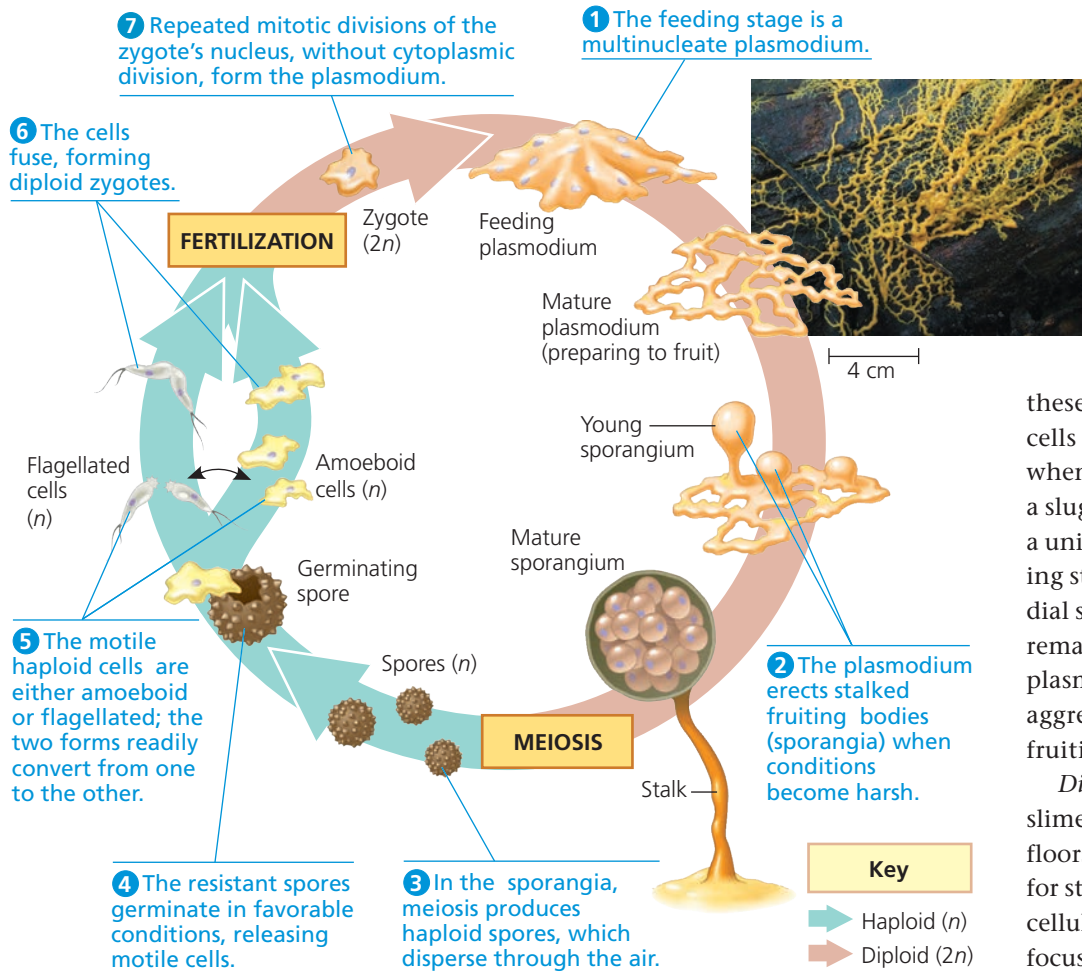


Conclusion The results show that Excavata, SAR, and Archaeplastida form a clade, which supports the hypothesis that the root of the tree is located between the unikonts and all other eukaryotes. Because support for this hypothesis is based on only one trait—the fusion of the genes for DHFR and TS—more data are needed to evaluate its validity.

Data from A. Stechmann and T. Cavalier-Smith, Rooting the eukaryote tree by using a derived gene fusion, *Science* 297:89–91 (2002).

WHAT IF? > Stechmann and Cavalier-Smith wrote that their conclusions are “valid only if the genes fused just once and were never secondarily split.” Why is this assumption critical to their approach?

▼ **Figure 28.25 A plasmodial slime mold.** The photograph shows a mature plasmodium, the feeding stage in the life cycle of a plasmodial slime mold. When food becomes scarce, the plasmodium forms stalked fruiting bodies that produce haploid spores that function in sexual reproduction.



these organisms consists of solitary cells that function individually, but when food is depleted, the cells form a slug-like aggregate that functions as a unit (Figure 28.26). Unlike the feeding stage (plasmodium) of a plasmodial slime mold, these aggregated cells remain separated by their individual plasma membranes. Ultimately, the aggregated cells form an asexual fruiting body.

Dictyostelium discoideum, a cellular slime mold commonly found on forest floors, has become a model organism for studying the evolution of multicellularity. One line of research has focused on the slime mold's fruiting body stage. During this stage, the cells that form the stalk die as they dry out,

Plasmodial Slime Molds Many plasmodial slime molds are brightly colored, often yellow or orange (Figure 28.25). As they grow, they form a mass called a plasmodium, which can be many centimeters in diameter. (Don't confuse a slime mold's plasmodium with the genus *Plasmodium*, which includes the parasitic apicomplexan that causes malaria.) Despite its size, the plasmodium is not multicellular; it is a single mass of cytoplasm that is undivided by plasma membranes and that contains many nuclei. This "supercell" is the product of mitotic nuclear divisions that are not followed by cytokinesis. The plasmodium extends pseudopodia through moist soil, leaf mulch, or rotting logs, engulfing food particles by phagocytosis as it grows. If the habitat begins to dry up or there is no food left, the plasmodium stops growing and differentiates into fruiting bodies that function in sexual reproduction.

Cellular Slime Molds The life cycle of the protists called cellular slime molds can prompt us to question what it means to be an individual organism. The feeding stage of

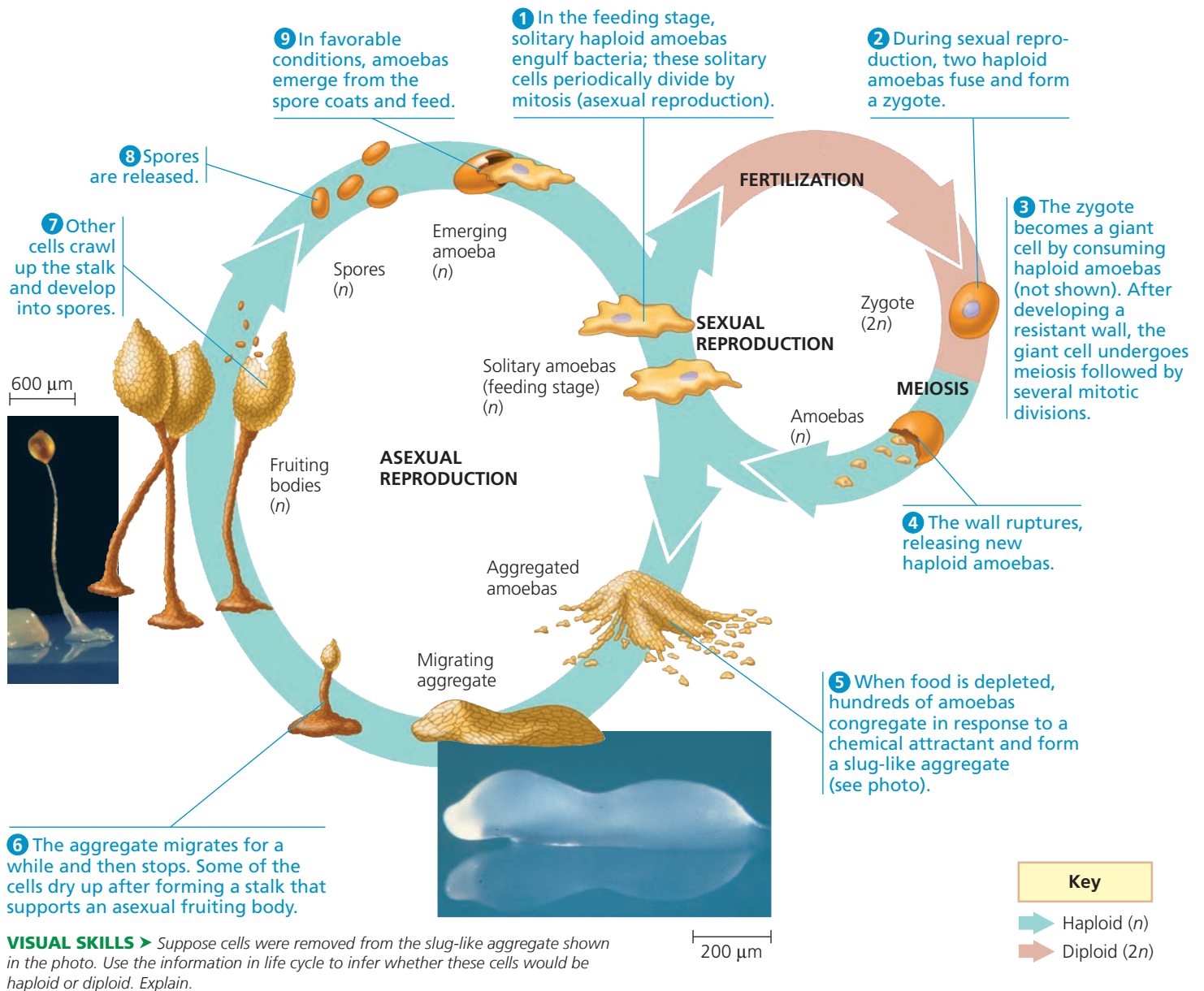
while the spore cells at the top survive and have the potential to reproduce (see Figure 28.26). Scientists have found that mutations in a single gene can turn individual *Dictyostelium* cells into "cheaters" that never become part of the stalk. Because these mutants gain a strong reproductive advantage over noncheaters, why don't all *Dictyostelium* cells cheat?

Recent discoveries suggest an answer to this question. Cheating cells lack a specific surface protein, and noncheating cells can recognize this difference. Noncheaters preferentially aggregate with other noncheaters, thus depriving cheaters of the chance to exploit them. Such a recognition system may have been important in the evolution of other multicellular eukaryotes, such as animals and plants.

Tubulinids

Tubulinids constitute a large and varied group of amoebozoans that have lobe- or tube-shaped pseudopodia. These unicellular protists are ubiquitous in soil as well as freshwater and marine environments. Most are heterotrophs that actively seek and consume bacteria and other protists; one such tubulinid

▼ **Figure 28.26** The life cycle of *Dictyostelium*, a cellular slime mold.



species, *Amoeba proteus*, is shown in Figure 28.2. Some tubulinids also feed on detritus (nonliving organic matter).

Entamoebas

Whereas most amoebozoans are free-living, those that belong to the genus *Entamoeba* are parasites. They infect all classes of vertebrate animals as well as some invertebrates. Humans are host to at least six species of *Entamoeba*, but only one, *E. histolytica*, is known to be pathogenic. *E. histolytica* causes amebic dysentery and is spread via contaminated drinking water, food, or eating utensils. Responsible for up to 100,000 deaths worldwide every year, the disease is the third-leading cause of death due to eukaryotic parasites, after malaria (see Figure 28.16) and schistosomiasis (see Figure 33.11).

Opisthokonts

Opisthokonts are an extremely diverse group of eukaryotes that includes animals, fungi, and several groups of protists. We will discuss the evolutionary history of fungi and animals in Chapters 31–34. Of the opisthokont protists, we will discuss the nucleariids in Chapter 31 because they are more closely related to fungi than they are to other protists. Similarly, we will discuss choanoflagellates in Chapter 32, since they are more closely related to animals than they are to other protists. The nucleariids and choanoflagellates illustrate why scientists have abandoned the former kingdom Protista: A monophyletic group that includes these single-celled eukaryotes would also have to include the multicellular animals and fungi that are closely related to them.

CONCEPT CHECK 28.5

1. Contrast the pseudopodia of amoebozoans and forams.
2. How does a plasmodial slime mold differ in structure from a cellular slime mold during the feeding stage?
3. **DRAW IT** > Recent evidence indicates that the root of the eukaryotic tree may lie between a clade that includes unikonts and excavates, and all other eukaryotes. Draw the tree suggested by this result.

For suggested answers, see Appendix A.

CONCEPT 28.6

Protists play key roles in ecological communities

Most protists are aquatic, and they are found almost anywhere there is water, including moist terrestrial habitats such as damp soil and leaf litter. In oceans, ponds, and lakes, many protists are bottom-dwellers that attach to rocks and other substrates or creep through the sand and silt. As we've seen, other protists are important constituents of plankton. We'll focus here on two key roles that protists play in the varied habitats in which they live: that of symbiont and that of producer.

Symbiotic Protists

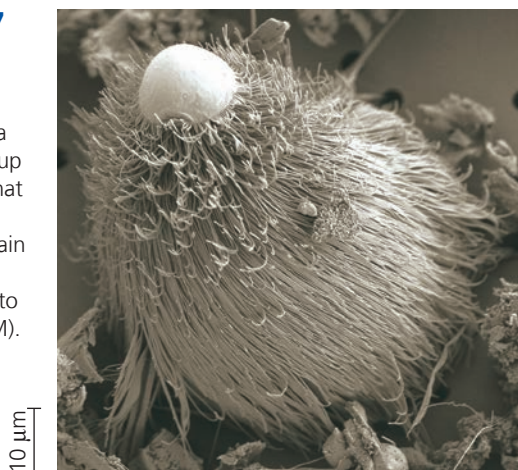
Many protists form symbiotic associations with other species. For example, photosynthetic dinoflagellates are food-providing symbiotic partners of the animals (coral polyps) that build coral reefs. Coral reefs are highly diverse ecological communities. That diversity ultimately depends on corals—and on the mutualistic protists that nourish them. Corals support reef diversity by providing food to some species and habitat to many others.

Another example is the wood-digesting protists that inhabit the gut of many termite species (Figure 28.27). Unaided, termites cannot digest wood, and they rely on protistan or prokaryotic symbionts to do so. Termites cause over \$3.5 billion in damage annually to wooden homes in the United States.

Symbiotic protists also include parasites that have compromised the economies of entire countries. Consider the

> Figure 28.27

A symbiotic protist. This organism is a hypermastigote, a member of a group of parabasalids that live in the gut of termites and certain cockroaches and enable the hosts to digest wood (SEM).



▼ **Figure 28.28 Sudden oak death.** Dead oak trees are visible in this landscape in Essex, England. Infected trees lose their ability to adjust to cycles of wet and dry weather.



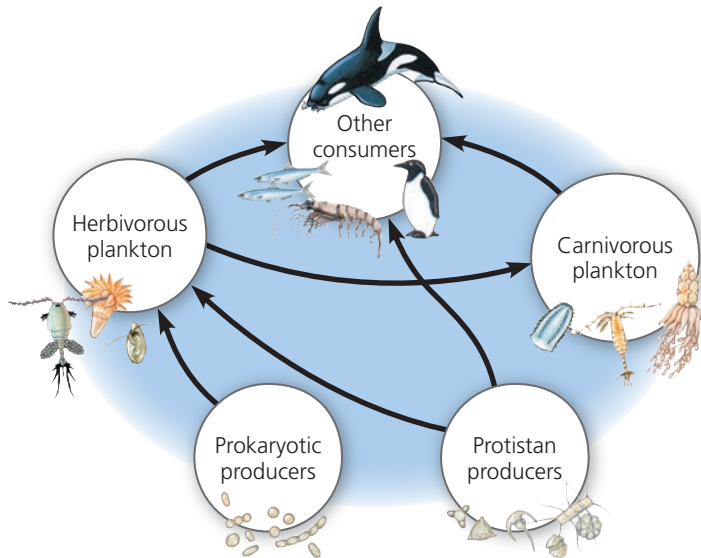
malaria-causing protist *Plasmodium*: Income levels in countries hard hit by malaria are 33% lower than in similar countries free of the disease. Protists can have devastating effects on other species too. Massive fish kills have been attributed to *Pfiesteria shumwayae* (see Figure 28.15), a dinoflagellate parasite that attaches to its victims and eats their skin. Among species that parasitize plants, the stramenopile *Phytophthora ramorum* has emerged as a major new forest pathogen. This species causes sudden oak death (SOD), a disease that has killed millions of oaks and other trees in the United States and Great Britain (Figure 28.28; also see Concept 54.5). A closely related species, *P. infestans*, causes potato late blight, which turns the stalks and stems of potato plants into black slime. Late blight contributed to the devastating Irish famine of the 19th century, in which a million people died and at least that many were forced to leave Ireland. The disease continues to be a major problem today, causing crop losses as high as 70% in some regions.

Photosynthetic Protists

Many protists are important **producers**, organisms that use energy from light (or in some prokaryotes, inorganic chemicals) to convert CO₂ to organic compounds. Producers form the base of ecological food webs. In aquatic communities, the main producers are photosynthetic protists and prokaryotes (Figure 28.29). All other organisms in the community depend on them for food, either directly (by eating them) or indirectly (by eating an organism that ate a producer). Scientists estimate that roughly 30% of the world's photosynthesis is performed by diatoms, dinoflagellates, multicellular algae, and other aquatic protists. Photosynthetic prokaryotes contribute another 20%, and plants are responsible for the remaining 50%.

Because producers form the foundation of food webs, factors that affect producers can dramatically affect their entire community. In aquatic environments, photosynthetic protists are often held in check by low concentrations of nitrogen, phosphorus, or iron. Various human actions can increase the

▼ **Figure 28.29 Protists: key producers in aquatic communities.** Arrows in this simplified food web lead from food sources to the organisms that eat them.



concentrations of these elements in aquatic communities. For example, when fertilizer is applied to a field, some of the fertilizer may be washed by rainfall into a river that drains into a lake or ocean. When people add nutrients to aquatic communities in this or other ways, the abundance of photosynthetic protists can increase spectacularly. Such increases can have major ecological consequences, including the formation of large “dead zones” in marine ecosystems (see Figure 56.23).

A pressing question is how global warming will affect photosynthetic protists and other producers. As shown in **Figure 28.30**, the growth and biomass of photosynthetic protists and prokaryotes have declined in many ocean

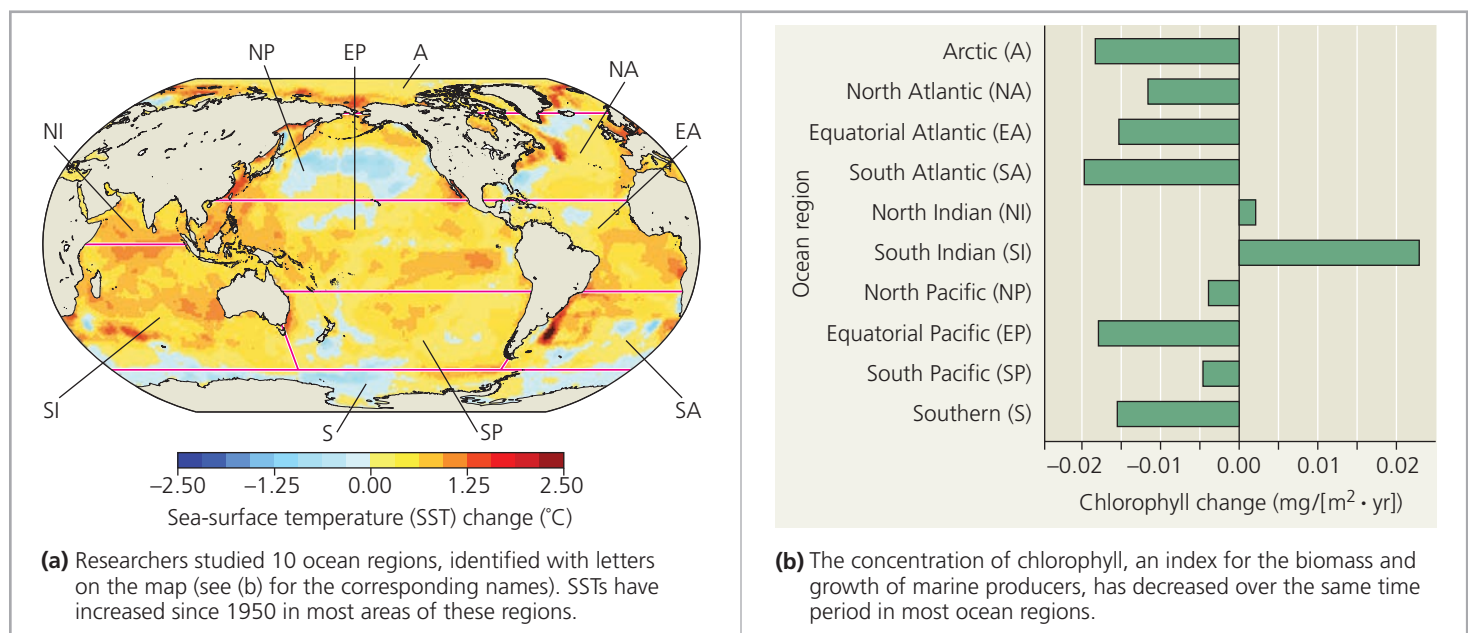
regions as sea surface temperatures have increased. By what mechanism do rising sea surface temperatures reduce the growth of marine producers? One hypothesis relates to the rise or upwelling of cold, nutrient-rich waters from below. Many marine producers rely on nutrients brought to the surface in this way. However, rising sea surface temperatures can cause the formation of a layer of light, warm water that acts as a barrier to nutrient upwelling—thus reducing the growth of marine producers. If sustained, the changes shown in Figure 28.30 would likely have far-reaching effects on marine ecosystems, fishery yields, and the global carbon cycle (see Figure 55.14). Global warming can also affect producers on land, but there the base of food webs is occupied not by protists but by plants, which we will discuss in Chapters 29 and 30.

CONCEPT CHECK 28.6

1. Justify the claim that photosynthetic protists are among the biosphere’s most important organisms.
2. Describe three symbioses that include protists.
3. **WHAT IF? >** High water temperatures and pollution can cause corals to expel their dinoflagellate symbionts. How might such “coral bleaching” affect corals and other species?
4. **MAKE CONNECTIONS >** The bacterium *Wolbachia* is a symbiont that lives in mosquito cells and spreads rapidly through mosquito populations. *Wolbachia* can make mosquitoes resistant to infection by *Plasmodium*; researchers are seeking a strain that confers resistance and does not harm mosquitoes. Compare evolutionary changes that could occur if malaria control is attempted using such a *Wolbachia* strain versus using insecticides to kill mosquitoes. (Review Figure 28.16 and Concept 23.4.)

For suggested answers, see Appendix A.

▼ **Figure 28.30 Effects of climate change on marine producers.**



28 Chapter Review



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SUMMARY OF KEY CONCEPTS

CONCEPT 28.1

Most eukaryotes are single-celled organisms (pp. 646–651)

- Domain Eukarya includes many groups of **protists**, along with plants, animals, and fungi. Unlike prokaryotes, protists and other eukaryotes have a nucleus and other membrane-enclosed organelles, as well as a cytoskeleton that enables them to have asymmetric forms and to change shape as they feed, move, or grow.
- Protists are structurally and functionally diverse and have a wide variety of life cycles. Most are unicellular. Protists include photoautotrophs, heterotrophs, and **mixotrophs**.



VOCAB
SELF-QUIZ
goo.gl/Rn5Uax

- Current evidence indicates that eukaryotes originated by **endosymbiosis** when an archaeal host (or a host closely related to the archaeans) engulfed an alpha proteobacterium that would evolve into an organelle found in all eukaryotes, the mitochondrion.
- Plastids are thought to be descendants of cyanobacteria that were engulfed by early eukaryotic cells. The plastid-bearing lineage eventually evolved into red algae and green algae. Other protist groups evolved from secondary endosymbiotic events in which red algae or green algae were themselves engulfed.
- In one hypothesis, eukaryotes are grouped into four supergroups, each a monophyletic clade: Excavata, SAR, Archaeplastida, and Unikonta.

? Describe similarities and differences between protists and other eukaryotes.

Key Concept/Eukaryote Supergroup	Major Groups	Key Morphological Characteristics	Specific Examples
CONCEPT 28.2 Excavates include protists with modified mitochondria and protists with unique flagella (pp. 651–653) ? What evidence indicates that the excavates form a clade?	Diplomonads and parabasalids Euglenozoans Kinetoplastids Euglenids	Modified mitochondria Spiral or crystalline rod inside flagella	<i>Giardia</i> , <i>Trichomonas</i> <i>Trypanosoma</i> , <i>Euglena</i>
CONCEPT 28.3 SAR is a highly diverse group of protists defined by DNA similarities (pp. 653–660) ? Although they are not photosynthetic, apicomplexan parasites such as Plasmodium have modified plastids. Describe a current hypothesis that explains this observation.	Stramenopiles Diatoms Golden algae Brown algae Alveolates Dinoflagellates Apicomplexans Ciliates Rhizarians Radiolarians Forams Cercozoans	Hairy and smooth flagella Membrane-enclosed sacs (alveoli) beneath plasma membrane Amoebas with threadlike pseudopodia	<i>Phytophthora</i> , <i>Laminaria</i> <i>Pfiesteria</i> , <i>Plasmodium</i> , <i>Paramecium</i> <i>Globigerina</i>
CONCEPT 28.4 Red algae and green algae are the closest relatives of plants (pp. 660–662) ? On what basis do systematists place plants in the same supergroup (Archaeplastida) as red and green algae?	Red algae Green algae Plants	Phycoerythrin (photosynthetic pigment) Plant-type chloroplasts (See Chapters 29 and 30.)	<i>Porphyra</i> <i>Chlamydomonas</i> , <i>Ulva</i> Mosses, ferns, conifers, flowering plants
CONCEPT 28.5 Unikonts include protists that are closely related to fungi and animals (pp. 662–666) ? Describe a key feature for each of the main protist subgroups of Unikonta.	Amoebozoans Slime molds Tubulinids Entamoebas Opisthokonts	Amoebas with lobe-shaped or tube-shaped pseudopodia (Highly variable; see Chapters 31–34.)	<i>Amoeba</i> , <i>Dictyostelium</i> Choanoflagellates, nucleariids, animals, fungi

CONCEPT 28.6

Protists play key roles in ecological communities (pp. 666–667)

- Protists form a wide range of mutualistic and parasitic relationships that affect their symbiotic partners and many other members of the community.
- Photosynthetic protists are among the most important **producers** in aquatic communities. Because they are at the base of the food web, factors that affect photosynthetic protists affect many other species in the community.



Describe several protists that are ecologically important.

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–6 can be found in the Study Area in MasteringBiology.

7. **EVOLUTION CONNECTION • DRAW IT** Medical researchers seek to develop drugs that can kill or restrict the growth of human pathogens yet have few harmful effects on patients. These drugs often work by disrupting the metabolism of the pathogen or by targeting its structural features.

Draw and label a phylogenetic tree that includes an ancestral prokaryote and the following groups of organisms: Excavata, SAR, Archaeplastida, Unikonta, and, within Unikonta, amoebozoans, animals, choanoflagellates, fungi, and nucleariids. Based on this tree, hypothesize whether it would be most difficult to develop drugs to combat human pathogens that are prokaryotes, protists, animals, or fungi. (You do not need to consider the evolution of drug resistance by the pathogen.)



PRACTICE TEST
goo.gl/iAsVgL

8. **SCIENTIFIC INQUIRY** Applying the “If... then” logic of deductive reasoning (see Concept 1.3), what are a few of the predictions that arise from the hypothesis that plants evolved from green algae? Put another way, how could you test this hypothesis?
9. **WRITE ABOUT A THEME: INTERACTIONS** Organisms interact with each other and the physical environment. In a short

essay (100–150 words), explain how the response of diatom populations to a drop in nutrient availability can affect both other organisms and aspects of the physical environment (such as carbon dioxide concentrations).

10. SYNTHESIZE YOUR KNOWLEDGE



This micrograph shows a single-celled eukaryote, the ciliate *Didinium* (left), about to engulf its *Paramecium* prey, which is also a ciliate. Identify the eukaryotic supergroup to which ciliates belong and describe the role of endosymbiosis in the evolutionary history of that supergroup. Are these ciliates more closely related to all other protists than they are to plants, fungi, or animals? Explain.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Nonvascular and Seedless Vascular Plants

29

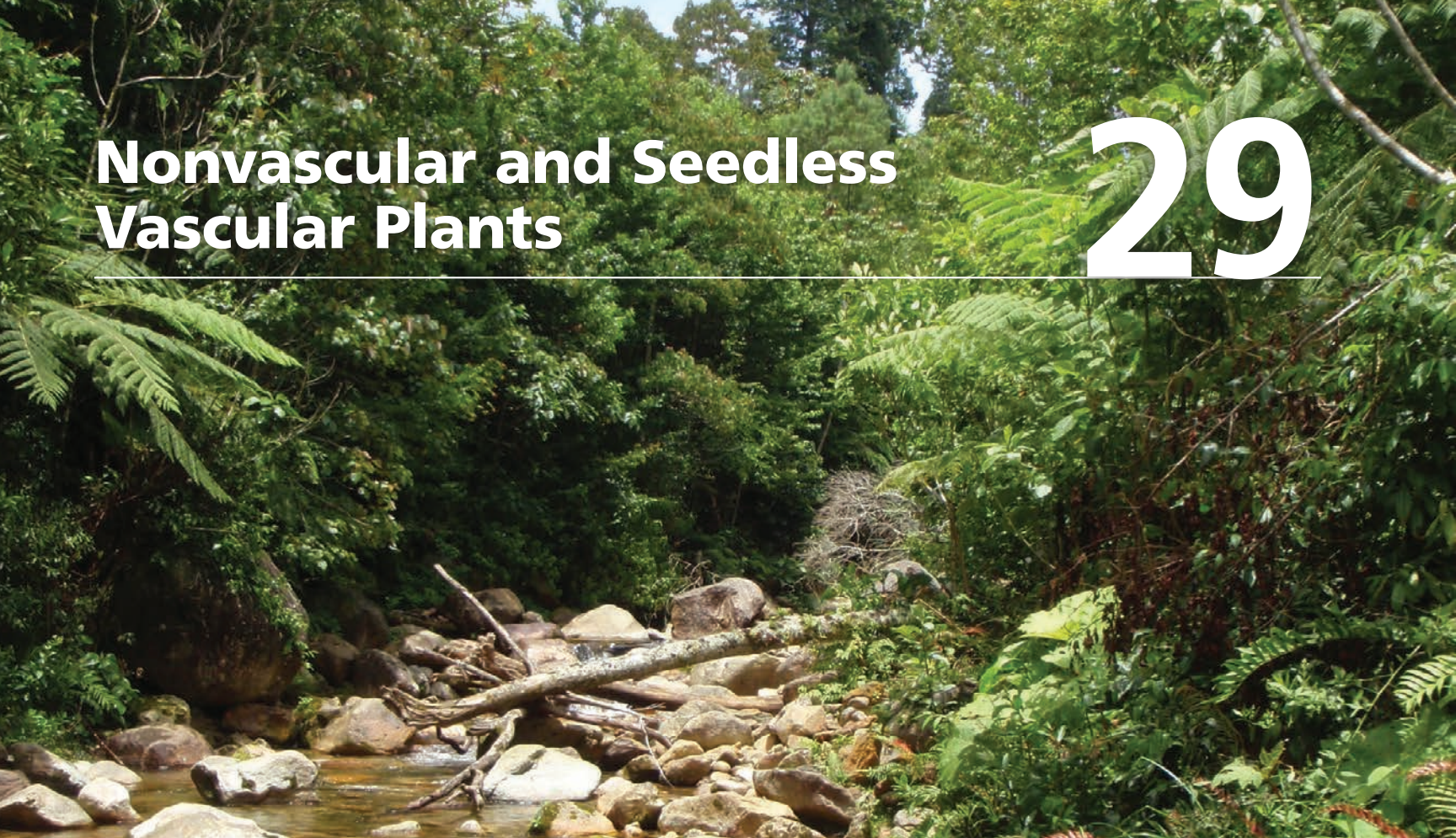


Figure 29.1 How did plants change the world?

KEY CONCEPTS

- 29.1** Plants evolved from green algae
- 29.2** Mosses and other nonvascular plants have life cycles dominated by gametophytes
- 29.3** Ferns and other seedless vascular plants were the first plants to grow tall



The Greening of Earth

Looking at a lush landscape, such as that shown in [Figure 29.1](#), it is hard to imagine the land without plants or other organisms. Yet for much of Earth's history, the land was largely lifeless. Geochemical analysis and fossil evidence suggest that thin coatings of cyanobacteria and protists existed on land by 1.2 billion years ago. But it was only within the last 500 million years that small plants, fungi, and animals joined them ashore. Finally, by about 385 million years ago, tall plants appeared, leading to the first forests.

Today, there are more than 290,000 known plant species. Plants inhabit all but the harshest environments, such as some mountaintop and desert areas and the polar ice sheets. Although a few plant species, such as sea grasses, returned to aquatic habitats during their evolution, most present-day plants live on land. In this text, we distinguish plants from algae, which are photosynthetic protists.

Plants enabled other life-forms to survive on land. For example, plants supply oxygen and are a key source of food for terrestrial animals. Also, by their very presence, plants such as the trees of a forest physically create the habitats required by animals and many other organisms. This chapter traces the first 100 million years of plant evolution, including the emergence of seedless plants such as mosses and ferns. Chapter 30 examines the later evolution of seed plants.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.

 **Get Ready for This Chapter**

CONCEPT 29.1

Plants evolved from green algae

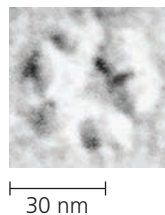
As you read in Chapter 28, green algae called charophytes are the closest relatives of plants. We'll begin with a closer look at the evidence for this relationship.

Morphological and Molecular Evidence

Many key traits of plants also appear in some algae. For example, plants are multicellular, eukaryotic, photosynthetic autotrophs, as are brown, red, and certain green algae. Plants have cell walls made of cellulose, and so do green algae, dinoflagellates, and brown algae. And chloroplasts with chlorophylls *a* and *b* are present in green algae, euglenids, and a few dinoflagellates, as well as in plants.

However, the charophytes are the only present-day algae that share the following distinctive traits with plants, suggesting that they are the closest living relatives of plants:

- **Rings of cellulose-synthesizing proteins.** The cells of both plants and charophytes have distinctive circular rings of proteins (small photo) embedded in the plasma membrane. These protein rings synthesize the cellulose microfibrils of the cell wall. In contrast, noncharophyte algae have linear sets of proteins that synthesize cellulose.
- **Structure of flagellated sperm.** In species of plants that have flagellated sperm, the structure of the sperm closely resembles that of charophyte sperm.
- **Formation of a phragmoplast.** Particular details of cell division occur only in plants and certain charophytes. For example, a group of microtubules known as the phragmoplast forms between the daughter nuclei of a dividing cell. A cell plate then develops in the middle of the phragmoplast, across the midline of the dividing cell (see Figure 12.10). The cell plate, in turn, gives rise to a new cross wall that separates the daughter cells.



Studies of nuclear, chloroplast, and mitochondrial DNA from a wide range of plants and algae indicate that certain groups of charophytes—such as *Zygnema* (see Figure 28.22a) and *Coleochaete*—are the closest living relatives of plants. Although this evidence shows that plants arose from within a group of charophyte algae, it does not mean that plants are descended from these living algae. Even so, present-day charophytes may tell us something about the algal ancestors of plants.

Adaptations Enabling the Move to Land

Many species of charophyte algae inhabit shallow waters around the edges of ponds and lakes, where they are subject to occasional drying. In such environments, natural selection favors individual algae that can survive periods when they are

not submerged. In charophytes, a layer of a durable polymer called **sporopollenin** prevents exposed zygotes from drying out. A similar chemical adaptation is found in the tough sporopollenin walls that encase plant spores.

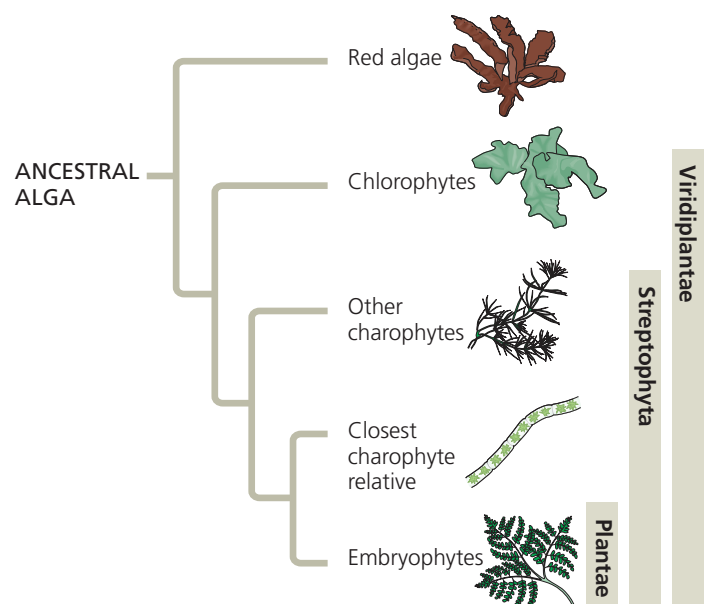
The accumulation of such traits by at least one population of charophyte algae (now extinct) probably enabled their descendants—the first plants—to live permanently above the waterline. This ability opened a new frontier: a terrestrial habitat that offered enormous benefits. The bright sunlight was unfiltered by water and plankton; the atmosphere offered more plentiful carbon dioxide than did water; and the soil by the water's edge was rich in some mineral nutrients. But these benefits were accompanied by challenges: a relative scarcity of water and a lack of structural support against gravity. (To appreciate why such support is important, picture how the soft body of a jellyfish sags when taken out of water.) Plants diversified as new adaptations arose that enabled them to thrive despite these challenges.

Today, what adaptations are unique to plants? The answer depends on where you draw the boundary dividing plants from algae (Figure 29.2). Since the placement of this boundary is the subject of ongoing debate, this text uses a traditional definition that equates the kingdom Plantae with embryophytes (plants with embryos). In this context, let's now examine the derived traits that separate plants from their closest algal relatives.

Derived Traits of Plants

Several adaptations that facilitate survival and reproduction on dry land emerged after plants diverged from their algal relatives. Figure 29.3 depicts five such traits that are found in plants but not in charophyte algae.

▼ Figure 29.2 Three possible “plant” kingdoms.



▼ Figure 29.3 Exploring Derived Traits of Plants

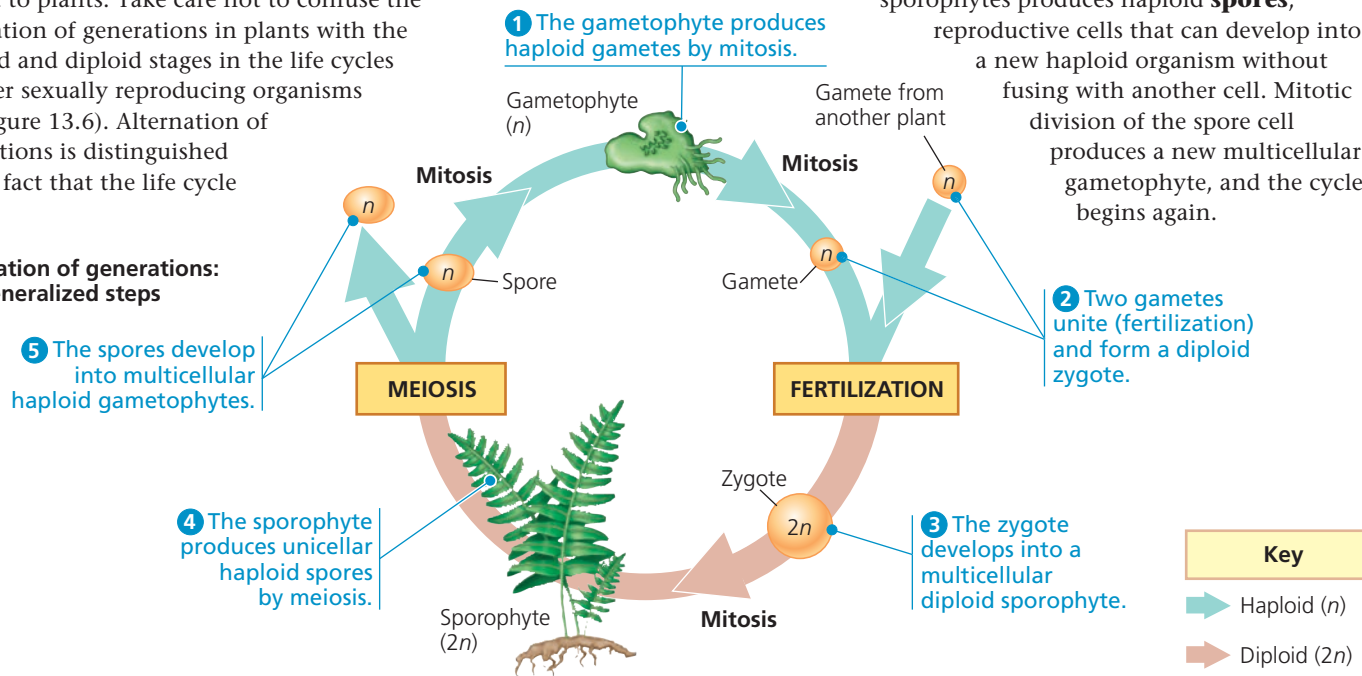
Charophyte algae lack the key traits of plants described in this figure: alternation of generations; multicellular, dependent embryos; walled spores produced in sporangia; multicellular gametangia; and apical meristems. This suggests that these traits were absent in the ancestor common to plants and charophytes but instead evolved as derived traits of plants. Not every plant exhibits all of these traits; certain lineages of plants have lost some traits over time.

Alternation of Generations

The life cycles of all plants alternate between two generations of distinct multicellular organisms: gametophytes and sporophytes. As shown in the diagram below (using a fern as an example), each generation gives rise to the other, a process that is called **alternation of generations**. This type of reproductive cycle evolved in various groups of algae but does not occur in the charophytes, the algae most closely related to plants. Take care not to confuse the alternation of generations in plants with the haploid and diploid stages in the life cycles of other sexually reproducing organisms (see Figure 13.6). Alternation of generations is distinguished by the fact that the life cycle

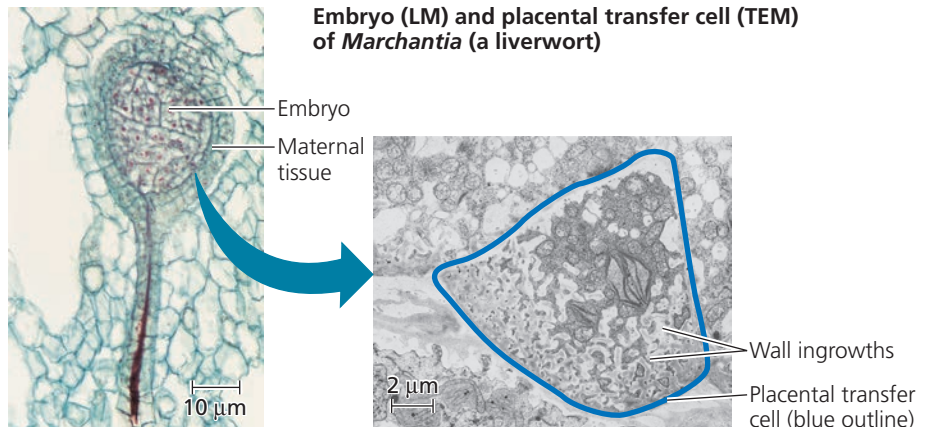
includes both multicellular haploid organisms and multicellular diploid organisms. The multicellular haploid **gametophyte** (“gamete-producing plant”) is named for its production by mitosis of haploid gametes—eggs and sperm—that fuse during fertilization, forming diploid zygotes. Mitotic division of the zygote produces a multicellular diploid **sporophyte** (“spore-producing plant”). Meiosis in a mature sporophyte produces haploid **spores**, reproductive cells that can develop into a new haploid organism without fusing with another cell. Mitotic division of the spore cell produces a new multicellular gametophyte, and the cycle begins again.

Alternation of generations:
five generalized steps



Multicellular, Dependent Embryos

As part of a life cycle with alternation of generations, multicellular plant embryos develop from zygotes that are retained within the tissues of the female parent (a gametophyte). The parental tissues protect the developing embryo from harsh environmental conditions and provide nutrients such as sugars and amino acids. The embryo has specialized *placental transfer cells* that enhance the transfer of nutrients to the embryo through elaborate ingrowths of the wall surface (plasma membrane and cell wall). The multicellular, dependent embryo of plants is such a significant derived trait that plants are also known as **embryophytes**.

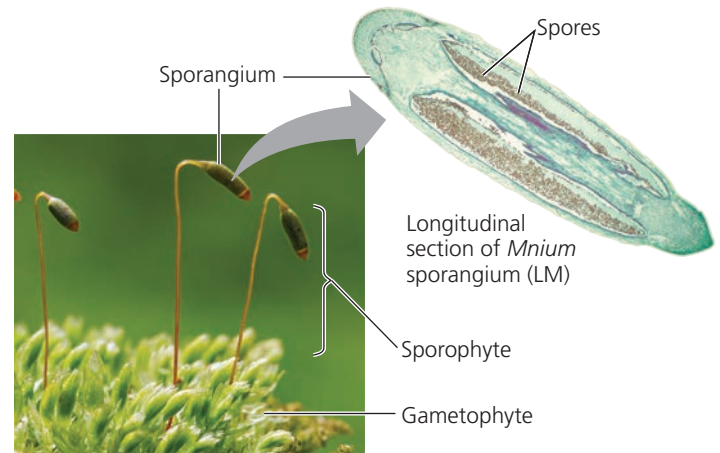


MAKE CONNECTIONS ► Review sexual life cycles in Figure 13.6. Identify which type of sexual life cycle has alternation of generations, and summarize how it differs from other life cycles.

Walled Spores Produced in Sporangia

Plant spores are haploid reproductive cells that can grow into multicellular haploid gametophytes by mitosis. The polymer sporopollenin makes the walls of plant spores tough and resistant to harsh environments. This chemical adaptation enables spores to be dispersed through dry air without harm.

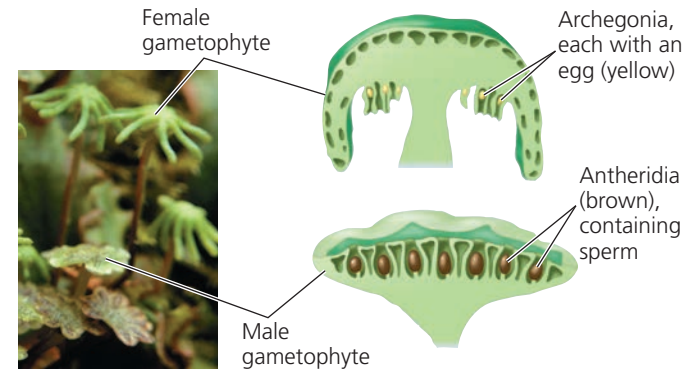
The sporophyte has multicellular organs called **sporangia** (singular, *sporangium*) that produce the spores. Within a sporangium, diploid cells called **sporocytes**, or spore mother cells, undergo meiosis and generate the haploid spores. The outer tissues of the sporangium protect the developing spores until they are released into the air. Multicellular sporangia that produce spores with sporopollenin-enriched walls are key terrestrial adaptations of plants. Although charophytes also produce spores, these algae lack multicellular sporangia, and their flagellated, water-dispersed spores lack sporopollenin.



Sporophytes and sporangia of *Mnium* (a moss)

Multicellular Gametangia

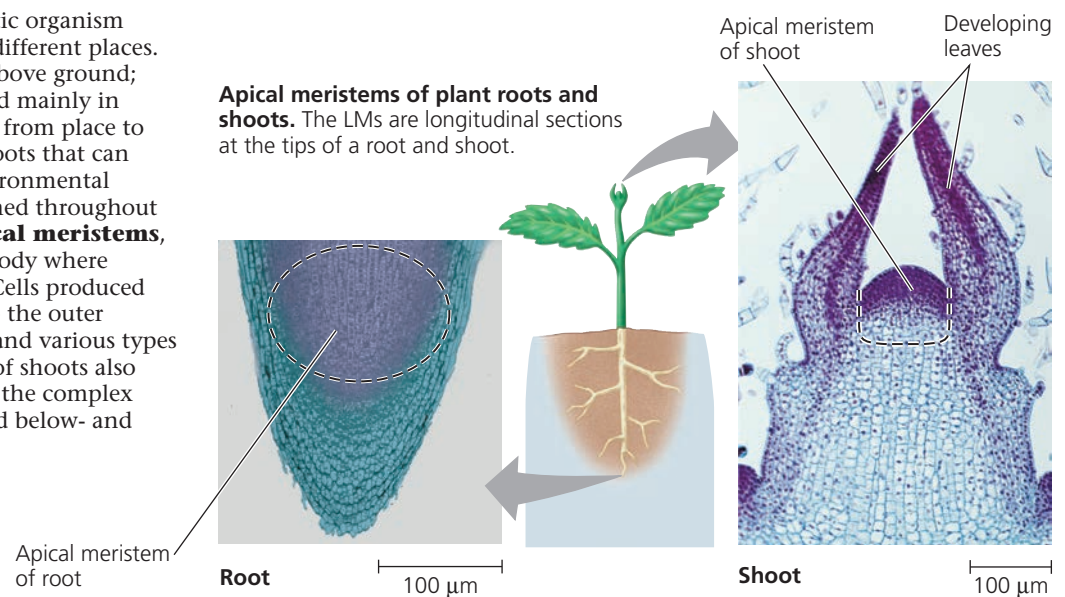
Another feature distinguishing early plants from their algal ancestors was the production of gametes within multicellular organs called **gametangia**. The female gametangia are called **archegonia** (singular, archegonium). Each archegonium is a pear-shaped organ that produces a single nonmotile egg retained within the bulbous part of the organ (the top for the species shown here). The male gametangia, called **antheridia** (singular, antheridium), produce sperm and release them into the environment. In many groups of present-day plants, the sperm have flagella and swim to the eggs through water droplets or a film of water. Each egg is fertilized within an archegonium, where the zygote develops into an embryo. The gametophytes of seed plants are so reduced in size (as you will see in Chapter 30) that the archegonia and antheridia have been lost in many lineages.



Archegonia and antheridia of *Marchantia* (a liverwort)

Apical Meristems

In terrestrial habitats, a photosynthetic organism finds essential resources in two very different places. Light and CO₂ are mainly available above ground; water and mineral nutrients are found mainly in the soil. Though plants cannot move from place to place, most plants have roots and shoots that can elongate, increasing exposure to environmental resources. Growth in length is sustained throughout the plant's life by the activity of **apical meristems**, regions at growing tips of the plant body where one or more cells divide repeatedly. Cells produced by apical meristems differentiate into the outer epidermis, which protects the body, and various types of internal tissues. Apical meristems of shoots also generate leaves in most plants. Thus, the complex bodies of most plants have specialized below- and aboveground organs.



Additional derived traits that relate to terrestrial life have evolved in many plant species. For example, the epidermis in many species has a covering, the **cuticle**, that consists of wax and other polymers. Permanently exposed to the air, plants run a far greater risk of desiccation (drying out) than do their algal relatives. The cuticle acts as waterproofing, helping prevent excessive water loss from the aboveground plant organs, while also providing some protection from microbial attack. Most plants also have specialized pores called **stomata** (singular, *stoma*), which support photosynthesis by allowing the exchange of CO₂ and O₂ between the outside air and the plant (see Figure 11.4). Stomata are also the main avenues by which water evaporates from the plant; in hot, dry conditions, the stomata close, minimizing water loss.

The earliest plants lacked true roots and leaves. Without roots, how did these plants absorb nutrients from the soil? Fossils dating from 420 million years ago reveal an adaptation that may have aided early plants in nutrient uptake: They formed symbiotic associations with fungi. We'll describe these associations, called *mycorrhizae*, and their benefits to both plants and fungi in more detail in Concept 31.1. For now, the main point is that mycorrhizal fungi form extensive networks of filaments through the soil and transfer nutrients to their symbiotic plant partner. This benefit may have helped plants without roots to colonize land.

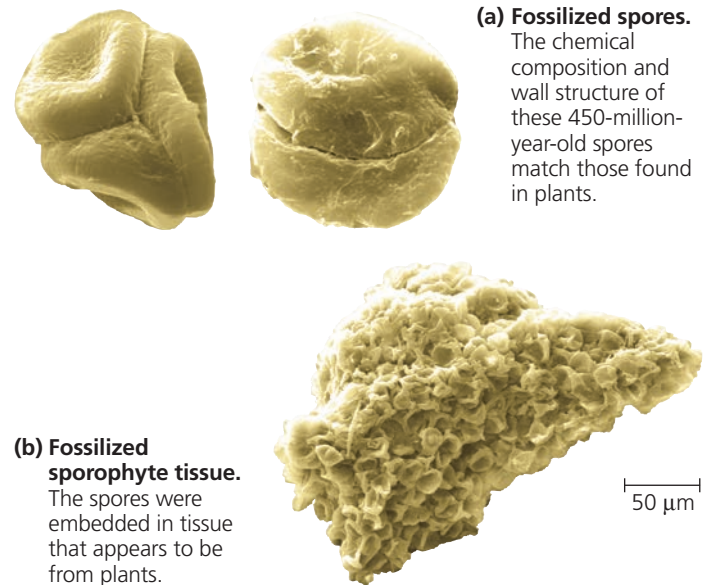
The Origin and Diversification of Plants

The algae most closely related to plants include many unicellular species and small colonial species. Since it is likely that the first plants were similarly small, the search for the earliest fossils of plants has focused on the microscopic world. As mentioned earlier, microorganisms colonized land as early as 1.2 billion years ago. But the microscopic fossils that document life on land changed dramatically 470 million years ago with the appearance of spores from early plants.

What distinguishes these spores from those of algae or fungi? One clue comes from their chemical composition, which matches the composition of plant spores today but differs from that of the spores of other organisms. In addition, the walls of these ancient spores have structural features that today are found only in the spores of certain plants (liverworts). And in rocks dating to 450 million years ago, researchers have discovered similar spores embedded in plant cuticle material that resembles spore-bearing tissue in living plants (**Figure 29.4**).

Fossils of larger plant structures, such as the *Cooksonia* sporangium in **Figure 29.5**, date to 425 million years ago, which is 45 million years

▼ **Figure 29.4 Ancient plant spores and tissue** (colorized SEMs).



after the appearance of plant spores in the fossil record. While the precise age (and form) of the first plants has yet to be discovered, those ancestral species gave rise to the vast diversity of living plants. **Table 29.1** summarizes the ten extant phyla in the taxonomic scheme used in this text. (Extant lineages are those that have surviving members.) As you read the rest of this section, look at Table 29.1 together with **Figure 29.6**, which reflects a view of plant phylogeny that is based on plant morphology, biochemistry, and genetics.

One way to distinguish groups of plants is whether or not they have an extensive system of **vascular tissue**, cells joined into tubes that transport water and nutrients throughout the plant body. Most present-day plants have a complex vascular tissue system and are therefore called **vascular plants**. Plants that do not have an extensive transport system—liverworts, mosses, and hornworts—are described as “nonvascular”

plants, even though some mosses do have simple vascular tissue. Nonvascular plants are often informally called **bryophytes** (from the Greek *bryon*, moss, and *phyton*, plant). Although the term *bryophyte* is commonly used to refer to all nonvascular plants, molecular studies and morphological analyses of sperm structure have concluded that bryophytes do not form a monophyletic group (a clade).

Vascular plants, which form a clade that comprises about 93% of all extant plant species, can be categorized further into smaller clades. Two of these clades are the **lycophytes** (the club mosses and their relatives) and the **monilophytes**



▲ **Figure 29.5 *Cooksonia* sporangium fossil**

Table 29.1 Ten Phyla of Extant Plants		
	Common Name	Number of Known Species
Nonvascular Plants (Bryophytes)		
Phylum Hepatophyta	Liverworts	9,000
Phylum Bryophyta	Mosses	15,000
Phylum Anthocerophyta	Hornworts	100
Vascular Plants		
Seedless Vascular Plants		
Phylum Lycophyta	Lycophytes	1,200
Phylum Monilophyta	Monilophytes	12,000
Seed Plants		
Gymnosperms		
Phylum Ginkgophyta	Ginkgo	1
Phylum Cycadophyta	Cycads	130
Phylum Gnetophyta	Gnetophytes	75
Phylum Coniferophyta	Conifers	600
Angiosperms		
Phylum Anthophyta	Flowering plants	250,000

(ferns and their relatives). The plants in each of these clades lack seeds, which is why collectively the two clades are often informally called **seedless vascular plants**. However,

notice in Figure 29.6 that, like bryophytes, seedless vascular plants do not form a clade.

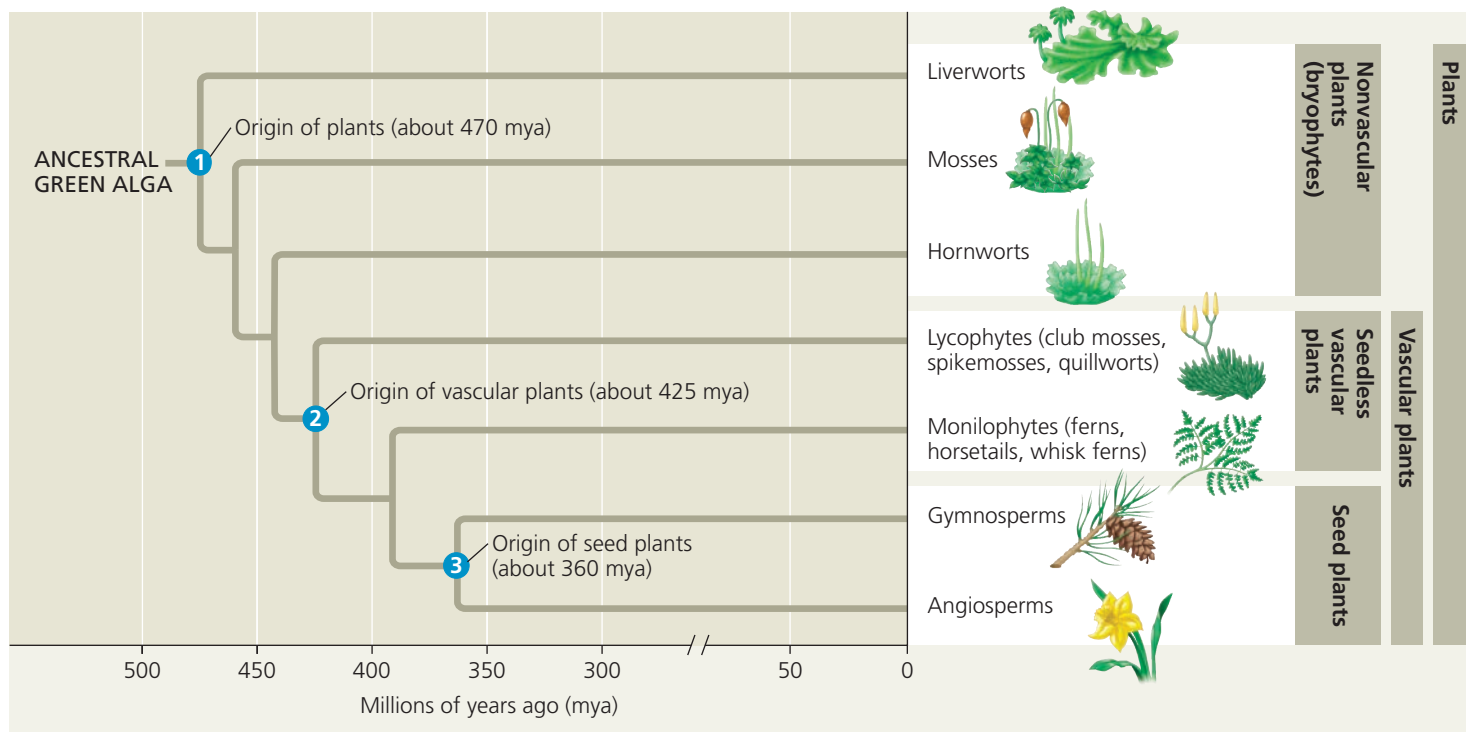
A group such as the bryophytes or the seedless vascular plants is sometimes referred to as a *grade*, a collection of organisms that share key biological features. Grades can be informative by grouping organisms according to their features, such as having a vascular system but lacking seeds. But members of a grade, unlike members of a clade, do not necessarily share the same ancestry. For example, even though monilophytes and lycophytes are all seedless vascular plants, monilophytes share a more recent common ancestor with seed plants. As a result, we would expect monilophytes and seed plants to share key traits not found in lycophytes—and they do, as you’ll read in Concept 29.3.

A third clade of vascular plants consists of seed plants, which represent the vast majority of living plant species. A **seed** is an embryo packaged with a supply of nutrients inside a protective coat. Seed plants can be divided into two groups, gymnosperms and angiosperms, based on the absence or presence of enclosed chambers in which seeds mature.

Gymnosperms (from the Greek *gymnos*, naked, and *sperm*, seed) are known as “naked seed” plants because their seeds are not enclosed in chambers. Living gymnosperm species, the most familiar of which are the conifers, form a clade.

Angiosperms (from the Greek *angion*, container) are a huge clade consisting of all flowering plants; their seeds develop

▼ **Figure 29.6 Highlights of plant evolution.** The phylogeny shown here illustrates a leading hypothesis about the relationships between plant groups.



MAKE CONNECTIONS ► The figure identifies which lineages are plants, nonvascular plants, vascular plants, seedless vascular plants, and seed plants. Which of these categories are monophyletic, and which are paraphyletic? Explain. (See Figure 22.10 to review these terms.)

inside chambers that originate within flowers. Nearly 90% of living plant species are angiosperms.

Note that the phylogeny depicted in Figure 29.6 focuses only on the relationships between extant plant lineages. Paleobotanists have also discovered fossils belonging to extinct plant lineages. As you'll read later in the chapter, these fossils can reveal intermediate steps in the emergence of plant groups found on Earth today.

CONCEPT CHECK 29.1

1. Why do researchers identify the charophytes rather than another group of algae as the closest living relatives of plants?
2. Identify four derived traits that distinguish plants from charophyte green algae *and* facilitate life on land. Explain.
3. **WHAT IF? >** What would the human life cycle be like if we had alternation of generations? Assume that the multicellular diploid stage would be similar in form to an adult human.

For suggested answers, see Appendix A.

CONCEPT 29.2

Mosses and other nonvascular plants have life cycles dominated by gametophytes



Nonvascular plants (bryophytes)

Seedless vascular plants
Gymnosperms
Angiosperms

The nonvascular plants (bryophytes) are represented today by three phyla of small, herbaceous

(nonwoody) plants: **liverworts** (phylum Hepatophyta), **mosses** (phylum Bryophyta), and **hornworts** (phylum Anthocerophyta). Liverworts and hornworts are named for their shapes, plus the suffix *wort* (from the Anglo-Saxon for “herb”). Mosses are familiar to many people, although some plants commonly called “mosses” are not really mosses at all. These include Irish moss (a red seaweed), reindeer moss (a lichen), club mosses (seedless vascular plants), and Spanish mosses (lichens in some regions and flowering plants in others).

Phylogenetic analyses indicate that liverworts, mosses, and hornworts diverged from other plant lineages early in the history of plant evolution (see Figure 29.6). Fossil evidence provides some support for this idea: The earliest spores of plants (dating from 450 to 470 million years ago) have structural features found only in the spores of liverworts, and by 430 million years ago spores similar to those of mosses and hornworts also occur in the fossil record. The earliest fossils of vascular plants date to about 425 million years ago.

Over the long course of their evolution, liverworts, mosses, and hornworts have acquired many unique adaptations. Next, we'll examine some of those features.

Bryophyte Gametophytes

Unlike vascular plants, in all three bryophyte phyla the haploid gametophytes are the dominant stage of the life cycle: They are usually larger and longer-living than the sporophytes, as shown in the moss life cycle in Figure 29.7. The sporophytes are typically present only part of the time.

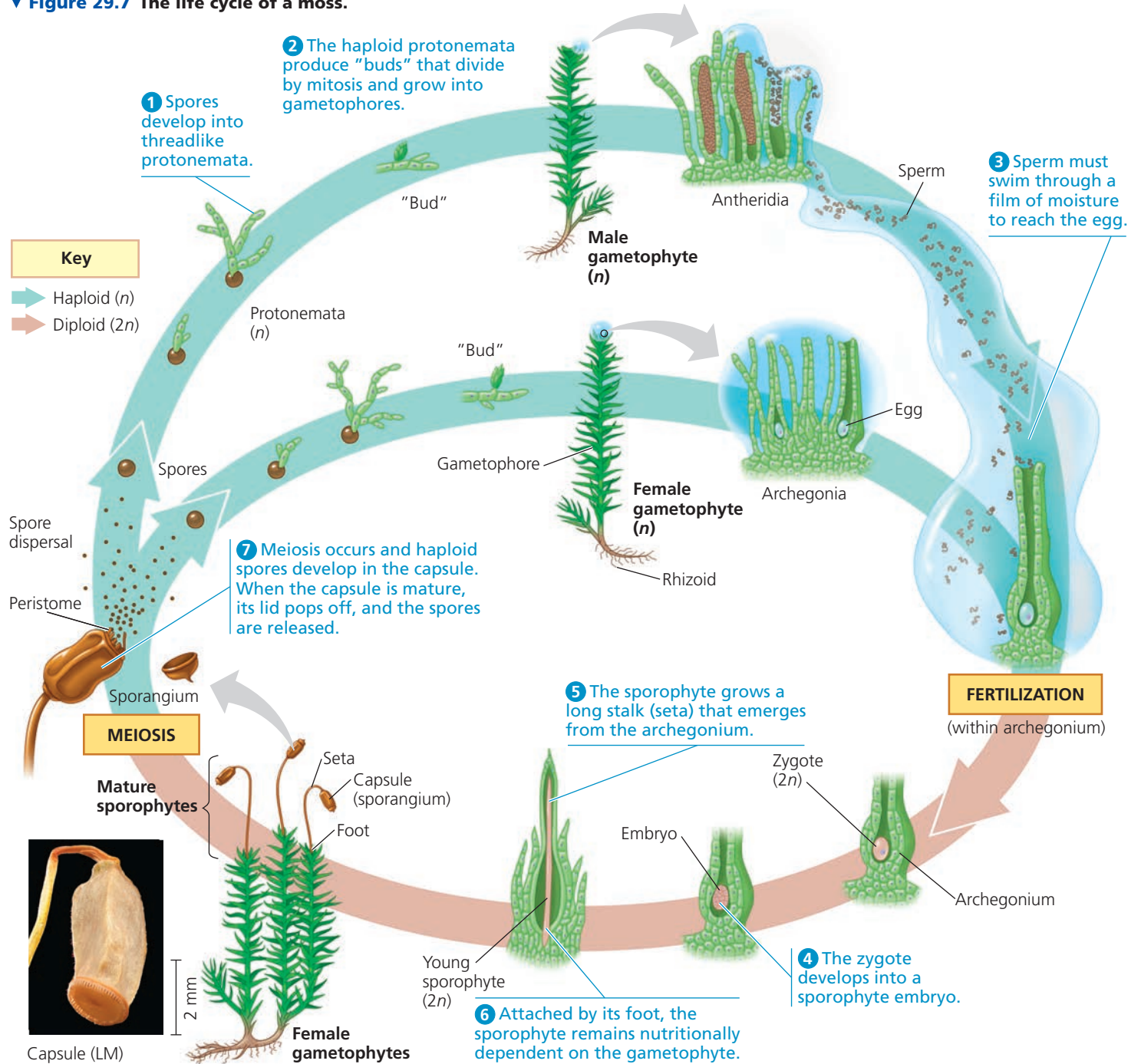
When bryophyte spores are dispersed to a favorable habitat, such as moist soil or tree bark, they may germinate and grow into gametophytes. Germinating moss spores, for example, characteristically produce a mass of green, branched, one-cell-thick filaments known as a **protonema** (plural, *protonemata*). A protonema has a large surface area that enhances absorption of water and minerals. In favorable conditions, a protonema produces one or more “buds.” (Note that when referring to nonvascular plants, we often use quotation marks for structures similar to the buds, stems, and leaves of vascular plants because the definitions of these terms are based on vascular plant organs.) Each of these bud-like growths has an apical meristem that generates a gamete-producing structure known as a **gametophore**. Together, a protonema and one or more gametophores make up the body of a moss gametophyte.

Bryophyte gametophytes generally form ground-hugging carpets, partly because their body parts are too thin to support a tall plant. A second constraint on the height of many bryophytes is the absence of vascular tissue, which would enable long-distance transport of water and nutrients. (The thin structure of bryophyte organs makes it possible to distribute materials for short distances without specialized vascular tissue.) However, some mosses have conducting tissues in the center of their “stems.” A few of these mosses can grow as tall as 60 cm (2 feet) as a result. Phylogenetic analyses suggest that conducting tissues similar to those of vascular plants arose independently in these mosses by convergent evolution.

The gametophytes are anchored by delicate **rhizoids**, which are long, tubular single cells (in liverworts and hornworts) or filaments of cells (in mosses). Unlike roots, which are found in vascular plant sporophytes, rhizoids are not composed of tissues. Bryophyte rhizoids also lack specialized conducting cells and do not play a primary role in water and mineral absorption.

Gametophytes can form multiple gametangia, each of which produces gametes and is covered by protective tissue. Each archegonium produces one egg, whereas each antheridium produces many sperm. Some bryophyte gametophytes are bisexual, but in mosses the archegonia and antheridia are

▼ **Figure 29.7** The life cycle of a moss.



VISUAL SKILLS ► In this diagram, does the sperm cell that fertilizes the egg cell differ genetically from the egg? Explain.

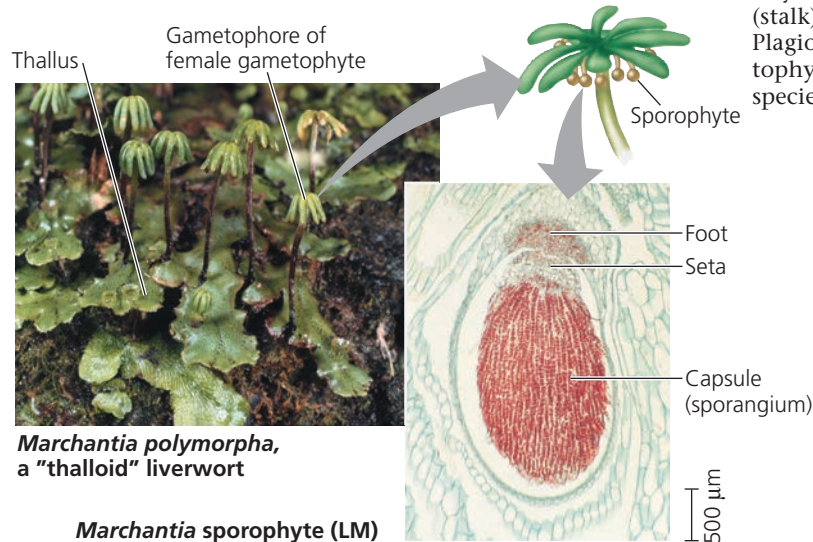
Animation: Moss Life Cycle

typically carried on separate female and male gametophytes. Flagellated sperm swim through a film of water toward eggs, entering the archegonia in response to chemical attractants. Eggs are not released but instead remain within the bases of archegonia. After fertilization, embryos are retained within the archegonia. Layers of placental transfer cells help transport nutrients to the embryos as they develop into sporophytes.

Bryophyte sperm typically require a film of water to reach the eggs. Given this requirement, it is not surprising that many bryophyte species are found in moist habitats. The fact that sperm swim through water to reach the egg also means that in species with separate male and female gametophytes (most species of mosses), sexual reproduction is likely to be more successful when individuals are located close to one another.

Liverworts (Phylum Hepatophyta)

This phylum's common and scientific names (from the Latin *hepaticus*, liver) refer to the liver-shaped gametophytes of its members, such as *Marchantia*, shown below. In medieval times, their shape was thought to be a sign that the plants could help treat

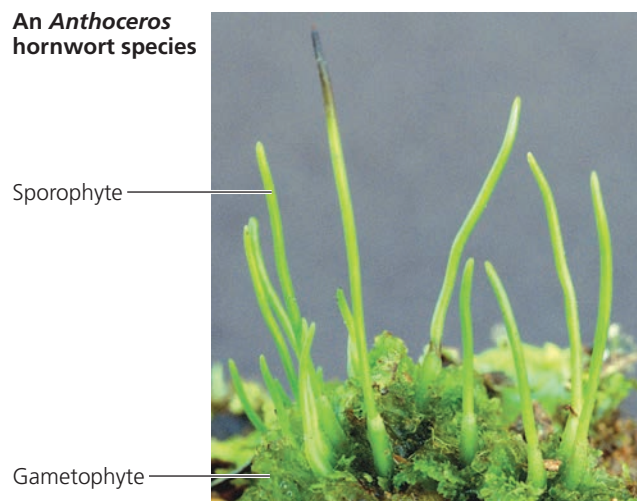


liver diseases. Some liverworts, including *Marchantia*, are described as "thalloid" because of the flattened shape of their gametophytes. *Marchantia* gametangia are elevated on gametophores that look like miniature trees. You would need a magnifying glass to see the sporophytes, which have a short seta (stalk) with an oval or round capsule. Other liverworts, such as *Plagiochila* are called "leafy" because their stemlike gametophytes have many leaflike appendages. There are many more species of leafy liverworts than thalloid liverworts.



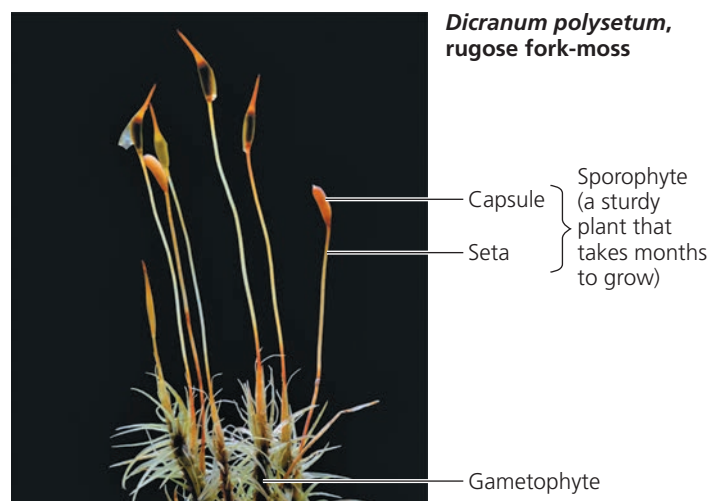
Hornworts (Phylum Anthocerophyta)

This phylum's common and scientific names (from the Greek *keras*, horn) refer to the long, tapered shape of the sporophyte. A typical sporophyte can grow to about 5 cm high. Unlike a liverwort or moss sporophyte, a hornwort sporophyte lacks a seta and consists only of a sporangium. The sporangium releases mature spores by splitting open, starting at the tip of the horn. The gametophytes, which are usually 1–2 cm in diameter, grow mostly horizontally and often have multiple sporophytes attached. Hornworts are frequently among the first species to colonize open areas with moist soils; a symbiotic relationship with nitrogen-fixing cyanobacteria contributes to their ability to do this (nitrogen is often in short supply in such areas).



Mosses (Phylum Bryophyta)

Moss gametophytes, which range in height from less than 1 mm up to 60 cm, are less than 15 cm tall in most species. The familiar carpet of moss you observe consists mainly of gametophytes. The blades of their "leaves" are usually only one cell thick, but more complex "leaves" that have ridges coated with cuticle can be found on the common hairy-cap moss (*Polytrichum*) and its close relatives. Moss sporophytes are typically elongated and visible to the naked eye, with heights ranging up to about 20 cm. Though green and photosynthetic when young, they turn tan or brownish red when ready to release spores.





Many bryophyte species can increase the number of individuals in a local area through various methods of asexual reproduction. For example, some mosses reproduce asexually by forming *brood bodies*, small plantlets (as shown here) that detach from the parent plant and grow into new, genetically identical copies of their parent.

Bryophyte Sporophytes

The cells of bryophyte sporophytes contain plastids that are usually green and photosynthetic when the sporophytes are young. Even so, bryophyte sporophytes cannot live independently. A bryophyte sporophyte remains attached to its parental gametophyte throughout the sporophyte's lifetime, dependent on the gametophyte for supplies of sugars, amino acids, minerals, and water.

Bryophytes have the smallest sporophytes of all extant plant groups, consistent with the hypothesis that larger sporophytes evolved only later, in the vascular plants. A typical bryophyte sporophyte consists of a foot, a seta, and a sporangium. Embedded in the archegonium, the **foot** absorbs nutrients from the gametophyte. The **seta** (plural, *setae*), or stalk, conducts these materials to the sporangium, also called a **capsule**, which produces spores by meiosis.

Bryophyte sporophytes can produce enormous numbers of spores. A single moss capsule, for example, can generate over 5 million spores. In most mosses, the seta becomes elongated, enhancing spore dispersal by elevating the capsule. Typically, the upper part of the capsule features a ring of interlocking, tooth-like structures known as the **peristome** (see Figure 29.7). These “teeth” open under dry conditions and close again when it is moist. This allows moss spores to be discharged gradually, via periodic gusts of wind that can carry them long distances.

Moss and hornwort sporophytes are often larger and more complex than those of liverworts. For example, hornwort sporophytes, which superficially resemble grass blades, have a cuticle. Moss and hornwort sporophytes also have stomata, as do all vascular plants (but not liverworts).

Figure 29.8 shows some examples of gametophytes and sporophytes in the bryophyte phyla.

The Ecological and Economic Importance of Mosses

Wind dispersal of lightweight spores has distributed mosses throughout the world. These plants are particularly common and diverse in moist forests and wetlands. Some mosses colonize bare, sandy soil, where, researchers have found, they help retain nitrogen in the soil. In northern coniferous forests, species such as the feather moss *Pleurozium*

harbor nitrogen-fixing cyanobacteria that increase the availability of nitrogen in the ecosystem. Other mosses inhabit such extreme environments as mountaintops, tundra, and deserts. Many mosses are able to live in very cold or dry habitats because they can survive the loss of most of their body water, then rehydrate when moisture is available. Few vascular plants can survive the same degree of desiccation. Moreover, phenolic compounds in moss cell walls absorb damaging levels of UV radiation present in deserts or at high altitudes.

One wetland moss genus, *Sphagnum*, or peat moss, is often a major component of deposits of partially decayed organic

▼ Figure 29.9

Inquiry Can bryophytes help prevent landslides on tropical mountains?

Field Study Tropical lowland forests are poor in bryophytes. Higher up on the mountains, however, in so-called mossy forests or cloud forests (because they are often shrouded in clouds), the soil, tree trunks, and branches are all laden with bryophytes due to lower temperatures and higher moisture. The tree-dwelling bryophytes as well as other plants and lichens are called epiphytes. An example of a mossy forest on a tropical mountain is shown below. Tamás Pócs, a Hungarian botanist with much field experience in the tropics, studied the epiphytic biomass and its water-holding capacity in the mossy forests on the Uluguru Mountains in southeastern Tanzania, Africa.



Results In the mossy forests on the Uluguru Mountains, the total epiphyte mass was found to be approximately 13.7 tonnes dry weight per hectare. The epiphytic bryophytes alone were able to absorb nearly 30,000 L (30 tonnes) of water per hectare during a single rain after a relatively dry period.

Conclusion The thick bryophyte cover on the trees and soil acts as a giant sponge to significantly alleviate the effect of torrential tropical rains, considerably reducing soil erosion, occurrence of landslides, and leaching of nutrients on the mountain slopes. The bryophytes allow the water to filter gradually to the soil, and they regulate the level of agriculturally important watercourses.

Source: T. Pócs, Tropical forest bryophytes, in A. J. E. Smith (editor), *Bryophyte Ecology*, 59–104 (Chapman & Hall, 1982).

WHAT IF? > The mountain slopes below mossy forests are often in agricultural use—for example, as coffee plantations. What would happen to these plantations if the mossy forests were felled?

material known as **peat** (Figure 29.10a). Boggy regions with thick layers of peat are called peatlands. *Sphagnum* does not decay readily, in part because of phenolic compounds embedded in its cell walls. The low temperature, pH, and oxygen level of peatlands also inhibit decay of moss and other organisms in these boggy wetlands. As a result, some peatlands have preserved corpses for thousands of years (Figure 29.10b).

Peat has long been a fuel source in Europe and Asia, and it is still harvested for fuel today, notably in Ireland and Canada. Peat moss is also useful as a soil conditioner and for packing plant roots during shipment because it has large dead cells that can absorb roughly 20 times the moss's weight in water.

Peatlands cover 3% of Earth's land surface and contain roughly 30% of the world's soil carbon: Globally, an estimated 450 billion tons of organic carbon is stored as peat. Current overharvesting of *Sphagnum*—primarily for use in peat-fired power stations—may contribute to global warming by releasing stored CO₂. In addition, if global temperatures continue to rise, the water levels of some peatlands are expected to drop. Such a change would expose peat to air and cause it to decompose, thereby releasing additional stored CO₂ and contributing

▼ **Figure 29.10** *Sphagnum*, or peat moss: a bryophyte with economic, ecological, and archaeological significance.



(a) Peat being harvested from a peatland



(b) "Tollund Man," a bog mummy dating from 405–100 B.C.E. The acidic, oxygen-poor conditions produced by *Sphagnum* can preserve human or other animal bodies for thousands of years.

further to global warming. The historical and expected future effects of *Sphagnum* on the global climate underscore the importance of preserving and managing peatlands.

Mosses may have a long history of affecting climate change. In the **Scientific Skills Exercise**, you will explore the question of whether they did so during the Ordovician period by contributing to the weathering of rocks.

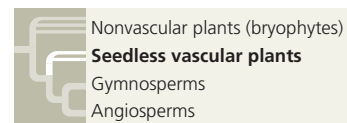
CONCEPT CHECK 29.2

1. How do bryophytes differ from other plants?
2. Explain why many bryophytes require a moist habitat to survive.
3. **MAKE CONNECTIONS** ▶ Review the discussion of feedback regulation in Concept 1.1. Could effects of global warming on peatlands alter CO₂ concentrations in ways that result in negative or positive feedback? Explain.

For suggested answers, see Appendix A.

CONCEPT 29.3

Ferns and other seedless vascular plants were the first plants to grow tall



During the first 100 million years of plant evolution, bryophytes were prominent types of vegetation. But it is

vascular plants that dominate most landscapes today. The earliest fossils of vascular plants date to 425 million years ago. These plants lacked seeds but had well-developed vascular systems, an evolutionary novelty that set the stage for vascular plants to grow taller than their bryophyte counterparts. As in bryophytes, however, the sperm of ferns and all other seedless vascular plants are flagellated and swim through a film of water to reach eggs. In part because of these swimming sperm, seedless vascular plants today are most common in damp environments.

Origins and Traits of Vascular Plants

Unlike the nonvascular plants, ancient relatives of vascular plants had branched sporophytes that were not dependent on gametophytes for nutrition (Figure 29.11). Although these early plants were less than 20 cm tall, their branching enabled their bodies to become more complex and to have multiple sporangia. As plant bodies became more complex over time, competition for space and sunlight probably increased. As we'll see, that competition may have stimulated still more evolution in vascular plants, eventually leading to the formation of the first forests.

Early vascular plants had some derived traits of today's vascular plants, but they lacked roots and some other adaptations that evolved later. The main traits that characterize living vascular plants are life cycles with dominant sporophytes,

SCIENTIFIC SKILLS EXERCISE

Making Bar Graphs and Interpreting Data

Could Nonvascular Plants Have Caused Weathering of Rocks and Contributed to Climate Change During the Ordovician Period? The oldest traces of terrestrial plants are fossilized spores formed 470 million years ago. Between that time and the end of the Ordovician period 444 million years ago, the atmospheric CO₂ level dropped by half, and the climate cooled dramatically.

One possible cause of the drop in CO₂ during the Ordovician period is the breakdown, or weathering, of rock. As rock weathers, calcium silicate (Ca₂SiCO₃) is released and combines with CO₂ from the air, producing calcium carbonate (CaCO₃). Today, the roots of vascular plants increase rock weathering and mineral release by producing acids that break down rock and soil. Although nonvascular plants lack roots, they require the same mineral nutrients as vascular plants. Could nonvascular plants also increase the chemical weathering of rock? If so, they could have contributed to the decline in atmospheric CO₂ during the Ordovician. In this exercise, you will interpret data from a study of the effects of moss on releasing minerals from two types of rock.

How the Experiment Was Done The researchers set up experimental and control microcosms, or small artificial ecosystems, to measure mineral release from rocks. First, they placed rock fragments of volcanic origin, either granite or andesite, into small glass containers. Then they mixed water and macerated (chopped and crushed) moss of the species *Physcomitrella patens*. They added this mixture to the experimental microcosms (72 granite and 41 andesite). For the control microcosms (77 granite and 37 andesite), they filtered out the moss and just added the water. After 130 days, they measured the amounts of various minerals found in the water in the control microcosms and in the water and moss in the experimental microcosms.

Data from the Experiment The moss grew (increased its biomass) in the experimental microcosms. The table shows the mean amounts in micromoles (μmol) of several minerals measured in the water and the moss in the microcosms.

	Ca ²⁺ (μmol)		Mg ²⁺ (μmol)		K ⁺ (μmol)	
	Granite	Andesite	Granite	Andesite	Granite	Andesite
Mean weathered amount released in water in the control microcosms	1.68	1.54	0.42	0.13	0.68	0.60
Mean weathered amount released in water in the experimental microcosms	1.27	1.84	0.34	0.13	0.65	0.64
Mean weathered amount taken up by moss in the experimental microcosms	1.09	3.62	0.31	0.56	1.07	0.28

Data from T. M. Lenton et al., First plants cooled the Ordovician, *Nature Geoscience* 5:86–89 (2012).

INTERPRET THE DATA

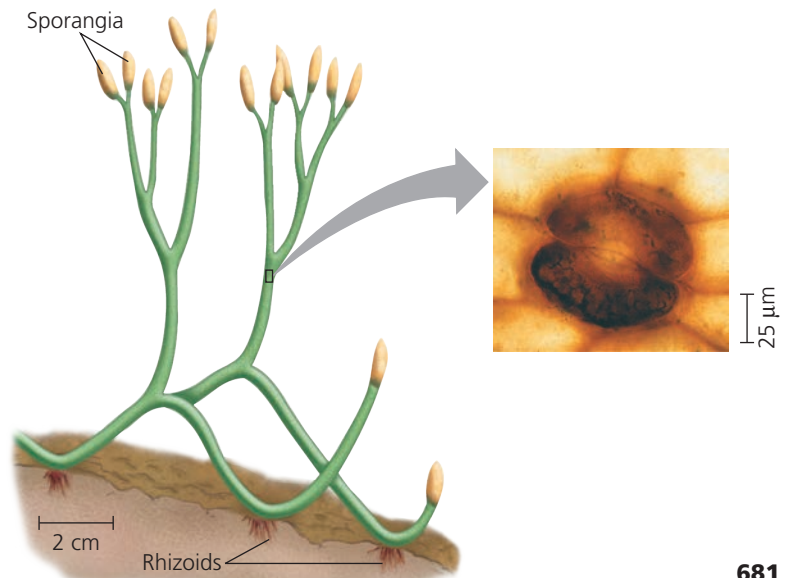
1. Why did the researchers add filtrate from which macerated moss had been removed to the control microcosms?
2. Make two bar graphs (for granite and andesite) comparing the mean amounts of each element weathered from rocks in the control and experimental microcosms. (Hint: For an experimental microcosm, what sum represents the total amount weathered from rocks?)
3. Overall, what is the effect of moss on chemical weathering of rock? Are the results similar or different for granite and andesite?
4. Based on their experimental results, the researchers added weathering of rock by nonvascular plants to simulation models of the Ordovician climate. The new models predicted decreased CO₂ levels and global cooling sufficient to produce the glaciations in the late Ordovician period. What assumptions did the researchers make in using results from their experiments in climate simulation models?
5. "Life has profoundly changed the Earth." Explain whether or not these experimental results support this statement.



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.



► **Figure 29.11 Sporophytes of *Aglaophyton major*, an ancient relative of living vascular plants.** This reconstruction from 405-million-year-old fossils exhibits dichotomous (Y-shaped) branching with sporangia at the ends of branches. Sporophyte branching characterizes living vascular plants but is lacking in living nonvascular plants (bryophytes). *Aglaophyton* had structures called rhizoids that anchored it to the ground. The inset shows a fossilized stoma of *A. major* (colorized LM).



transport in vascular tissues called xylem and phloem, and well-developed roots and leaves, including spore-bearing leaves called sporophylls.

Life Cycles with Dominant Sporophytes

As mentioned earlier, mosses and other bryophytes have life cycles dominated by gametophytes (see Figure 29.7). Fossil evidence suggests that a change began to develop in some of the earliest vascular plants, whose gametophytes and sporophytes were about equal in size. Further reductions in gametophyte size occurred among extant vascular plants; in these groups, the sporophyte generation is the larger and more complex form in the alternation of generations (Figure 29.12). In ferns, for example, the familiar leafy plants are the sporophytes. You would have to get down on your hands and knees and search the ground carefully to find fern gametophytes, which are tiny structures that often grow on or just below the soil surface.

Transport in Xylem and Phloem

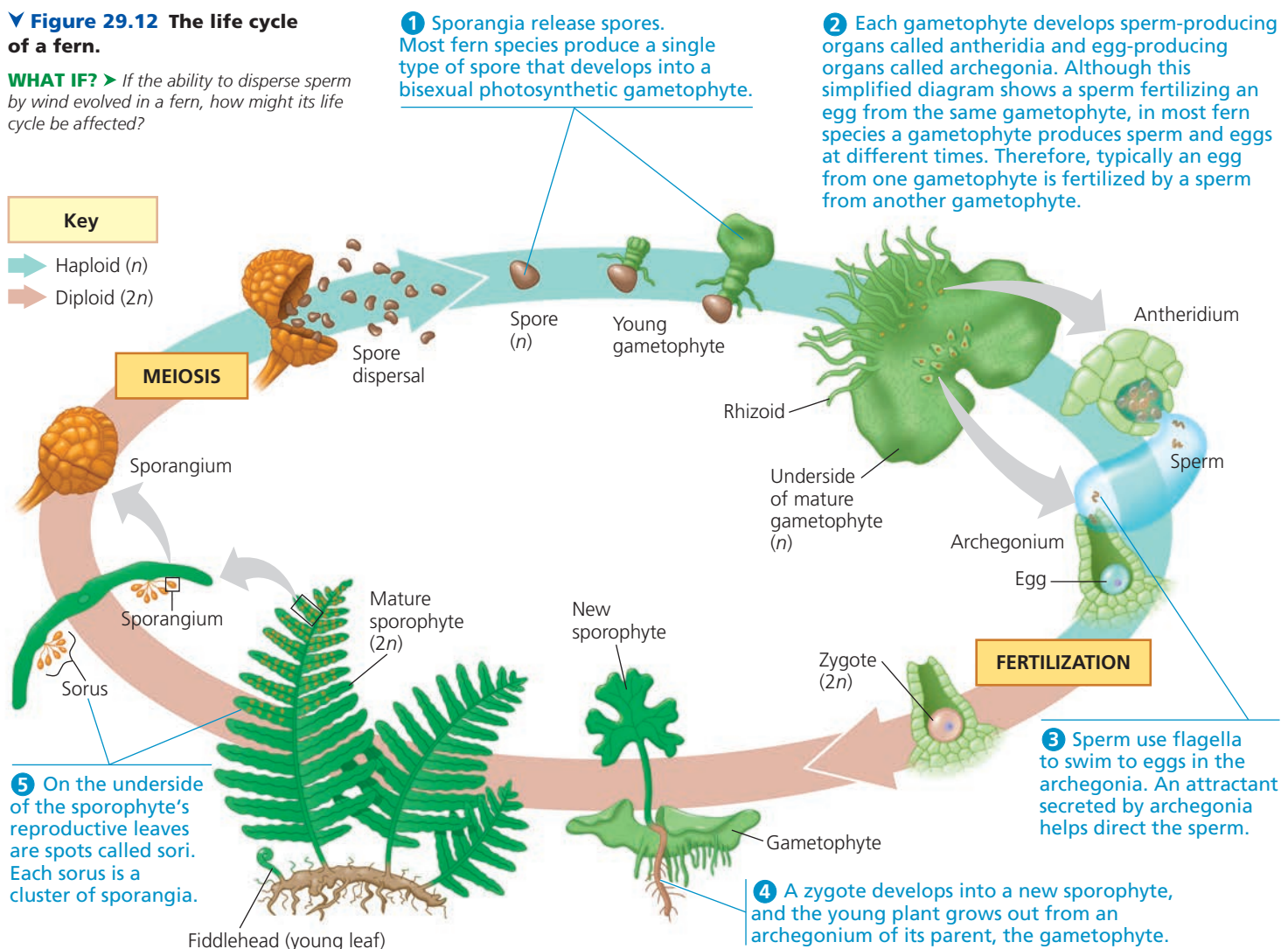
Vascular plants have two types of vascular tissue: xylem and phloem. **Xylem** conducts most of the water and minerals.

The xylem of most vascular plants includes **tracheids**, tube-shaped cells that carry water and minerals up from the roots (see Figure 35.10). The water-conducting cells in vascular plants are *lignified*; that is, their cell walls are strengthened by the polymer **lignin**. The tissue called **phloem** has cells arranged into tubes that distribute sugars, amino acids, and other organic products (see Figure 35.10).

Lignified vascular tissue helped enable vascular plants to grow tall. Their stems became strong enough to provide support against gravity, and they could transport water and mineral nutrients high above the ground. Tall plants could also outcompete short plants for access to the sunlight needed for photosynthesis. In addition, the spores of tall plants could disperse farther than those of short plants, enabling tall species to colonize new environments rapidly. Overall, the ability to grow tall gave vascular plants a competitive edge over nonvascular plants, which typically are less than 5 cm in height. Competition among vascular plants also would have increased, leading to selection for taller growth forms—a process that eventually gave rise to the trees that formed the first forests about 385 million years ago.

▼ **Figure 29.12** The life cycle of a fern.

WHAT IF? > If the ability to disperse sperm by wind evolved in a fern, how might its life cycle be affected?



Evolution of Roots

Vascular tissue also provides benefits below ground. Instead of the rhizoids seen in bryophytes, roots evolved in the sporophytes of almost all vascular plants. **Roots** are organs that absorb water and nutrients from the soil. Roots also anchor vascular plants to the ground, hence allowing the shoot system to grow taller.

Root tissues of living plants closely resemble stem tissues of early vascular plants preserved in fossils. This suggests that roots may have evolved from the lowest belowground portions of stems in ancient vascular plants. It is unclear whether roots evolved only once in the common ancestor of all vascular plants or independently in different lineages. Although the roots of living members of these lineages of vascular plants share many similarities, fossil evidence hints at convergent evolution. The oldest fossils of lycophytes, for example, already displayed simple roots 400 million years ago, when the ancestors of ferns and seed plants still had none. Studying genes that control root development in different vascular plant species may help resolve this question.

Evolution of Leaves

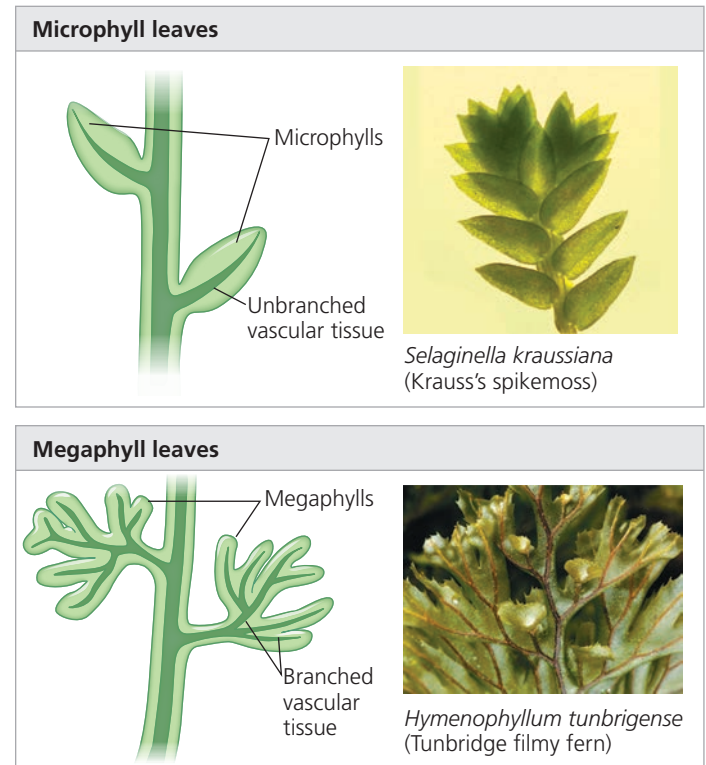
Leaves are structures that serve as the primary photosynthetic organ of vascular plants. In terms of size and complexity, leaves can be classified as either microphylls or megaphylls (Figure 29.13). All of the lycophytes—and only the lycophytes—have **microphylls**, small, often spine-shaped leaves supported by a single strand of vascular tissue. Almost all other vascular plants have **megaphylls**, leaves with a highly branched vascular system; a few species have reduced leaves that appear to have evolved from megaphylls. Megaphylls are typically larger than microphylls and therefore support greater photosynthetic productivity than microphylls. Microphylls first appear in the fossil record 410 million years ago, but megaphylls do not emerge until about 370 million years ago, toward the end of the Devonian period.

Sporophylls and Spore Variations

One milestone in the evolution of plants was the emergence of **sporophylls**, modified leaves that bear sporangia. Sporophylls vary greatly in structure. For example, fern sporophylls produce clusters of sporangia known as **sori** (singular, *sorus*), usually on the undersides of the sporophylls (see Figure 29.12). In many lycophytes and in most gymnosperms, groups of sporophylls form cone-like structures called **strobili**. The sporophylls of angiosperms are called *carpels* and *stamens* (see Figure 30.8).

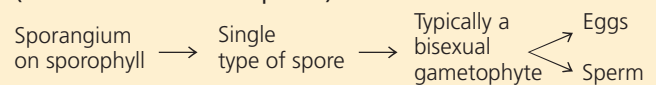
Most seedless vascular plant species are **homosporous**: They have one type of sporophyll bearing one type of sporangium that produces one type of spore, which typically develops into a bisexual gametophyte, as in most ferns. In contrast, a **heterosporous** species has two types of sporophylls: megasporophylls and microsporophylls. Megasporophylls have

▼ **Figure 29.13** Microphyll and megaphyll leaves.

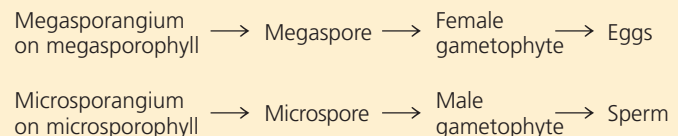


megasporangia, which produce **megaspores**, spores that develop into female gametophytes. Microsporophylls have microsporangia, which produce **microspores**, smaller spores that develop into male gametophytes. All seed plants and a few seedless vascular plants are heterosporous. The following diagram compares the two conditions:

Homosporous spore production (most seedless vascular plants)



Heterosporous spore production (all seed plants)



Classification of Seedless Vascular Plants

As we noted earlier, biologists recognize two clades of living seedless vascular plants: the lycophytes (phylum Lycophyta) and the monilophytes (phylum Monilophyta). The lycophytes include the club mosses, the spikemosses, and the quillworts. The monilophytes include the ferns, the horsetails, and the whisk ferns and their relatives. Although ferns, horsetails,

Lycophytes (Phylum Lycophyta)

Many lycophytes grow on tropical trees as epiphytes, plants that use other plants as a substrate but are not parasites. Other species grow on temperate forest floors. In some species, the tiny gametophytes live above ground and are photosynthetic. Others live below ground, nurtured by symbiotic fungi.

Sporophytes have upright stems with many small leaves, as well as ground-hugging stems that produce dichotomously branching roots. Spike mosses are usually relatively small and often grow horizontally. In many club mosses and spike mosses, sporophylls are clustered into club-shaped cones (strobili). Quillworts, named for their leaf shape, form a single genus whose members live in marshy areas or as submerged aquatic plants. Club mosses are all homosporous, whereas spike mosses and quillworts are all heterosporous. The spores of club mosses are released in clouds and are so rich in oil that magicians and photographers once ignited them to create smoke or flashes of light.

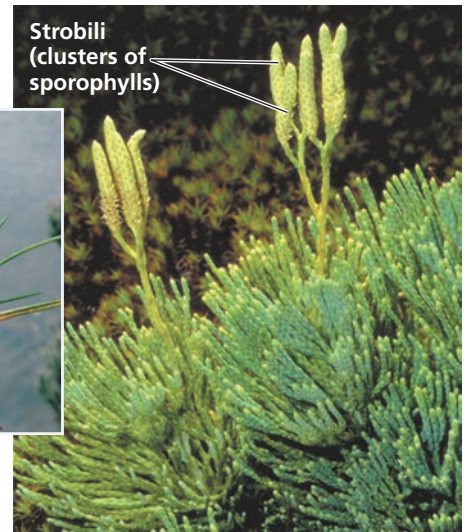
Selaginella moellendorffii, a spike moss



Isoetes gunnii, a quillwort



Strobili (clusters of sporophylls)



Diphasiastrum tristachyum, a club moss

Monilophytes (Phylum Monilophyta)



Matteuccia struthiopteris (ostrich fern)

Ferns

Unlike the lycophytes, ferns have megaphylls (see Figure 29.13). The sporophytes typically have horizontal stems that give rise to large leaves called fronds, often divided into leaflets. A frond grows as its coiled tip, the fiddlehead, unfurls.

Almost all species are homosporous. The gametophyte in some species shrivels and dies after the young sporophyte detaches itself. In most species, sporophytes have stalked sporangia with springlike devices that catapult spores several meters. Airborne spores can be carried far from their origin. Some species produce more than a trillion spores in a plant's lifetime.



Equisetum telmateia, giant horsetail

Horsetails

The group's name refers to the brushy appearance of the stems, which have a gritty texture that made them historically useful as "scouring rushes" for pots and pans. Some species have separate fertile (cone-bearing) and vegetative stems. Horsetails are homosporous, with cones releasing spores that typically give rise to bisexual gametophytes.

Horsetails are also called arthrophytes ("jointed plants") because their stems have joints. Rings of small leaves or branches emerge from each joint, but the stem is the main photosynthetic organ. Large air canals carry oxygen to the roots, which often grow in waterlogged soil.



Psilotum nudum, a whisk fern

Whisk Ferns and Relatives

Like primitive vascular plant fossils, the sporophytes of whisk ferns (genus *Psilotum*) have dichotomously branching stems but no roots. Stems have scalelike outgrowths that lack vascular tissue and may have resulted from the evolutionary reduction of leaves. Each yellow knob on a stem consists of three fused sporangia. Species of the genus *Tmesipteris* closely related to whisk ferns and found only in the South Pacific, also lack roots but have small, leaflike outgrowths in their stems, giving them a vine-like appearance. Both genera are homosporous, with spores giving rise to bisexual gametophytes that grow underground and are only about a centimeter long.

and whisk ferns differ greatly in appearance, recent anatomical and molecular comparisons provide convincing evidence that these three groups make up a clade. Accordingly, many systematists now classify them together as the phylum Monilophyta, as we do in this chapter. Others refer to these groups as three separate phyla within a clade. **Figure 29.14** describes the two main groups of seedless vascular plants.

Phylum Lycophyta: **Club Mosses, Spikemosses, and Quillworts**

Present-day species of lycophytes are relicts of a far more impressive past. By the Carboniferous period (359–299 million years ago), the lycophyte evolutionary lineage included small herbaceous plants and giant trees with diameters of more than 2 m and heights of more than 40 m. The giant lycophyte trees thrived for millions of years in moist swamps, but their diversity declined when Earth’s climate became drier during the Permian period (299–252 million years ago). The small lycophytes survived, represented today by about 1,200 species. Though some are commonly called club mosses and spike-mosses, they are not true mosses (which, as discussed earlier, are nonvascular plants).

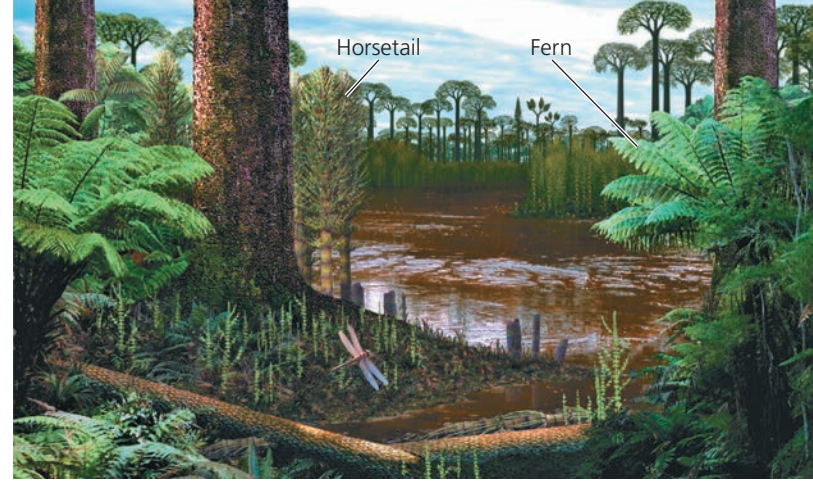
Phylum Monilophyta: **Ferns, Horsetails, and Whisk Ferns and Relatives**

Ferns radiated extensively from their Devonian origins and grew alongside lycophyte trees and horsetails in the great Carboniferous swamp forests. Today, ferns are by far the most widespread seedless vascular plants, numbering more than 12,000 species. Though most diverse in the tropics, many ferns thrive in temperate forests, and some species are even adapted to arid habitats.

As mentioned earlier, ferns and other monilophytes are more closely related to seed plants than to lycophytes. As a result, monilophytes and seed plants share traits that are not found in lycophytes, including megaphyll leaves and roots that can branch at various points along the length of an existing root. In lycophytes, by contrast, roots branch only at the growing tip of the root, forming a Y-shaped structure.

The monilophytes called horsetails were very diverse during the Carboniferous period, some growing as tall as 15 m. Today, only 15 species survive as a single, widely distributed genus, *Equisetum*, often found in marshy places and along streams.

Psilotum (whisk ferns) and a closely related genus, *Tmesipteris*, form a clade consisting mainly of tropical epiphytes. Plants in these two genera, the only vascular plants lacking true roots, once were called “living fossils” because of their resemblance to fossils of ancient relatives of living vascular plants (see Figures 29.11 and 29.14). However, much evidence, including analyses of DNA sequences and sperm structure, indicates that the genera *Psilotum* and *Tmesipteris*



▲ Figure 29.15 Artist's conception of a Carboniferous forest based on fossil evidence. Lycophyte trees, with trunks covered with small leaves, thrived in the “coal forests” of the Carboniferous, along with giant ferns and horsetails.

are closely related to ferns. This hypothesis suggests that their ancestor’s true roots were lost during evolution. Today, plants in these two genera absorb water and nutrients through numerous absorptive rhizoids.

The Significance of Seedless Vascular Plants

The ancestors of living lycophytes, horsetails, and ferns, along with their extinct seedless vascular relatives, grew to great heights during the Devonian and early Carboniferous, forming the first forests (**Figure 29.15**). How did their dramatic growth affect Earth and its other life?

One major effect was that early forests contributed to a large drop in CO₂ levels during the Carboniferous period, causing global cooling that resulted in widespread glacier formation. The trees of early forests contributed to this drop in CO₂ levels in part by the actions of their roots. The roots of vascular plants secrete acids that break down rocks, thereby increasing the rate at which calcium and magnesium are released from rocks into the soil. These chemicals react with carbon dioxide dissolved in rain water, forming compounds that ultimately wash into the oceans, where they are incorporated into rocks (calcium or magnesium carbonates). The net effect of these processes—which were accelerated by plants—is that CO₂ removed from the air is stored in marine rocks. Although carbon stored in these rocks can be returned to the atmosphere, it typically takes millions of years for this to occur (as when geological uplift brings the rocks to the surface, exposing them to erosion).

In addition, the seedless vascular plants that formed the first forests eventually became coal, again removing CO₂ from the atmosphere for long periods of time. In the stagnant waters of Carboniferous swamps, the dead bodies of early trees did not completely decay. This organic material turned to thick layers of peat, later covered by the sea. Marine sediments piled on top, and over millions of years, heat and pressure converted the peat to coal. In fact, Carboniferous coal deposits are the most extensive ever formed. Coal was crucial to the Industrial

Revolution, and people worldwide still burn 6 billion tons a year. It is ironic that coal, formed from plants that contributed to a global cooling, now contributes to global warming by returning carbon to the atmosphere (see Figure 56.29).

Growing along with the seedless plants in Carboniferous swamps were primitive seed plants. Though seed plants were not dominant at that time, they rose to prominence after the swamps began to dry up at the end of the Carboniferous period. The next chapter traces the origin and diversification of seed plants, continuing our story of adaptation to life on land.

CONCEPT CHECK 29.3

1. List the key derived traits found in monilophytes and seed plants, but not in lycophytes.
2. How do the main similarities and differences between seedless vascular plants and nonvascular plants affect function in these plants?
3. **MAKE CONNECTIONS** > In Figure 29.12, if fertilization occurred between gametes from one gametophyte, how would this affect the production of genetic variation from sexual reproduction? See Concept 13.4.

For suggested answers, see Appendix A.

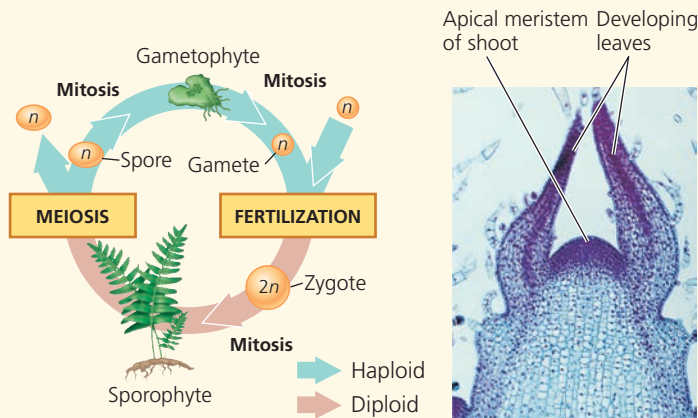
29 Chapter Review

SUMMARY OF KEY CONCEPTS

CONCEPT 29.1

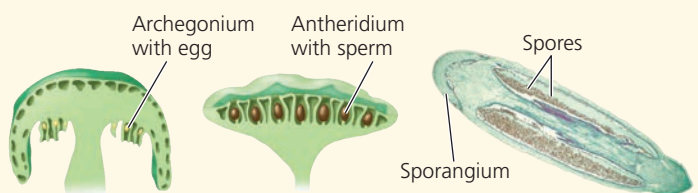
Plants evolved from green algae
(pp. 671–676)

- Morphological and biochemical traits, as well as similarities in nuclear and chloroplast genes, indicate that certain groups of charophytes are the closest living relatives of plants.
- A protective layer of **sporopollenin** and other traits allow charophytes to tolerate occasional drying along the edges of ponds and lakes. Such traits may have enabled the algal ancestors of plants to survive in terrestrial conditions, opening the way to the colonization of dry land.
- Derived traits that distinguish plants from charophytes, their closest algal relatives, include **cuticles**, **stomata**, multicellular dependent embryos, and the four shown here:



1 Alternation of generations

2 Apical meristems



3 Multicellular gametangia

4 Walled spores in sporangia

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- Fossils show that plants arose more than 470 million years ago. Subsequently, plants diverged into several major groups, including nonvascular plants (**bryophytes**); **seedless vascular plants**, such as **lycophytes** and ferns; and the two groups of seed plants: **gymnosperms** and **angiosperms**.

? Draw a phylogenetic tree illustrating our current understanding of plant phylogeny; label the common ancestor of plants and the origins of multicellular gametangia, vascular tissue, and seeds.

CONCEPT 29.2

Mosses and other nonvascular plants have life cycles dominated by gametophytes

(pp. 676–680)

- Lineages leading to the three extant clades of nonvascular plants, or **bryophytes**—**liverworts**, **mosses**, and **hornworts**—diverged from other plants early in plant evolution.
- In bryophytes, the dominant generation consists of haploid **gametophytes**, such as those that make up a carpet of moss. **Rhizoids** anchor gametophytes to the substrate on which they grow. The flagellated sperm produced by **antheridia** require a film of water to travel to the eggs in the **archegonia**.
- The diploid stage of the life cycle—the **sporophytes**—grow out of archegonia and are attached to the gametophytes and dependent on them for nourishment. Smaller and simpler than vascular plant sporophytes, they typically consist of a **foot**, **seta** (stalk), and **sporangium**.
- *Sphagnum*, or peat moss, is common in large regions known as peatlands and has many practical uses, including as a fuel.

? Summarize the ecological importance of mosses.

CONCEPT 29.3

Ferns and other seedless vascular plants were the first plants to grow tall (pp. 680–686)

- Fossils of the forerunners of today's vascular plants date back about 425 million years and show that these small plants had independent, branching sporophytes and a vascular system.
- Over time, other derived traits of living vascular plants arose, such as a life cycle with dominant sporophytes, lignified vascular tissue, well-developed **roots** and **leaves**, and **sporophylls**.
- Seedless vascular plants include the **lycophytes** (phylum Lycopphyta: club mosses, spikemosses, and quillworts) and

the **monilophytes** (phylum Monilophyta: ferns, horsetails, and whisk ferns and relatives). Current evidence indicates that seedless vascular plants, like bryophytes, do not form a clade.

- Ancient lineages of lycophytes included both small herbaceous plants and large trees. Present-day lycophytes are small herbaceous plants.
- Seedless vascular plants formed the earliest forests about 385 million years ago. Their growth may have contributed to a major global cooling that took place during the Carboniferous period. The decaying remnants of the first forests eventually became coal.

? What trait(s) allowed vascular plants to grow tall, and why might increased height have been advantageous?

TEST YOUR UNDERSTANDING



Multiple-choice Self-Quiz questions 1–5 can be found in the Study Area in MasteringBiology.

6. Identify each of the following structures as haploid or diploid.
- | | |
|----------------|-----------------|
| (A) sporophyte | (C) gametophyte |
| (B) spore | (D) zygote |



PRACTICE TEST
goo.gl/AsVgL

7. EVOLUTION CONNECTION

DRAW IT Draw a phylogenetic tree that represents our current understanding of evolutionary relationships between a moss, a gymnosperm, a lycophyte, and a fern. Use a charophyte alga as the outgroup. (See Figure 22.5 to review phylogenetic trees.) Label each branch point of the phylogeny with at least one derived character unique to the clade descended from the common ancestor represented by the branch point.

8. SCIENTIFIC INQUIRY

INTERPRET THE DATA The feather moss *Pleurozium schreberi* harbors species of symbiotic nitrogen-fixing bacteria. Scientists studying this moss in northern forests found that the percentage of the ground surface “covered” by the moss increased from about 5% in forests that burned 35 to 41 years ago to about 70% in forests that burned 170 or more years ago. From mosses growing in these forests, they also obtained the following data on nitrogen fixation:

Age (years after fire)	N fixation rate [kg N/(ha · yr)]
35	0.001
41	0.005
78	0.08
101	0.3
124	0.9
170	2.0
220	1.3
244	2.1
270	1.6
300	3.0
355	2.3

Data from O. Zackrisson et al., Nitrogen fixation increases with successional age in boreal forests, *Ecology* 85:3327–3334 (2006).

- (a) Use the data to draw a line graph, with age on the x-axis and the nitrogen fixation rate on the y-axis.
- (b) Along with the nitrogen added by nitrogen fixation, about 1 kg of nitrogen per hectare per year is deposited into northern forests from the atmosphere as rain and small particles. Evaluate the extent to which *Pleurozium* affects nitrogen availability in northern forests of different ages.

9. **WRITE ABOUT A THEME: INTERACTIONS** Giant lycophyte trees had microphylls, whereas ferns and seed plants have megaphylls. Write a short essay (100–150 words) describing how a forest of lycophyte trees may have differed from a forest of large ferns or seed plants. In your answer, consider how the type of forest may have affected interactions among small plants growing beneath the tall ones.

10. SYNTHESIZE YOUR KNOWLEDGE



These stomata are from the leaf of a common horsetail. Describe how stomata and other adaptations facilitated life on land and ultimately led to the formation of the first forests.

For selected answers, see Appendix A.



For additional practice questions, check out the **Dynamic Study Modules** in MasteringBiology. You can use them to study on your smartphone, tablet, or computer anytime, anywhere!

Seed Plants

30



▲ **Figure 30.1** How could these plants have reached this remote location?

KEY CONCEPTS

- 30.1** Seeds and pollen grains are key adaptations for life on land
- 30.2** Gymnosperms bear “naked” seeds, typically on cones
- 30.3** The reproductive adaptations of angiosperms include flowers and fruits
- 30.4** Human welfare depends on seed plants

▼ Fireweed seed



Transforming the World

On May 18, 1980, Mount St. Helens erupted with a force 500 times that of the Hiroshima atomic bomb. Traveling at over 300 miles per hour, the blast destroyed hundreds of hectares of forest, leaving the region covered in ash and devoid of visible life. Within a few years, however, plants such as fireweed (*Chamerion angustifolium*) had colonized the barren landscape (**Figure 30.1**).

Fireweed and other early arrivals reached the blast zone as seeds. A **seed** consists of an embryo and its food supply, surrounded by a protective coat. When mature, seeds are dispersed from their parent by wind or other means, enabling them to colonize distant locations.

Plants not only have affected the recovery of regions such as Mount St. Helens but also have transformed Earth. Continuing the saga of how this occurred, this chapter follows the emergence and diversification of the group to which fireweed belongs, the seed plants. Fossils and comparative studies of living plants offer clues about the origin of seed plants some 360 million years ago. As this new group became established, it dramatically altered the course of plant evolution. Indeed, seed plants have become the dominant producers on land, and they make up the vast majority of plant biodiversity today.

In this chapter, we will first examine the general features of seed plants. Then we will look at their evolutionary history and enormous impact on human society.

When you see this blue icon, log in to **MasteringBiology** and go to the Study Area for digital resources.

 **Get Ready for This Chapter**

CONCEPT 30.1

Seeds and pollen grains are key adaptations for life on land

We begin with an overview of terrestrial adaptations that seed plants added to those already present in nonvascular plants (bryophytes) and seedless vascular plants (see Concept 29.1). In addition to seeds, all seed plants have reduced gametophytes, heterospory, ovules, and pollen. As we'll see, these adaptations helped seed plants cope with conditions such as drought and exposure to ultraviolet (UV) radiation in sunlight. They also freed seed plants from requiring water for fertilization, enabling reproduction under a broader range of conditions than in seedless plants.

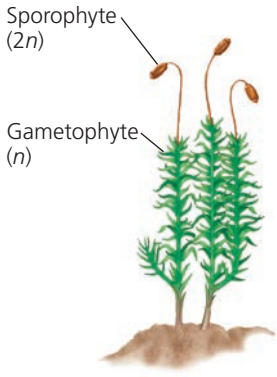
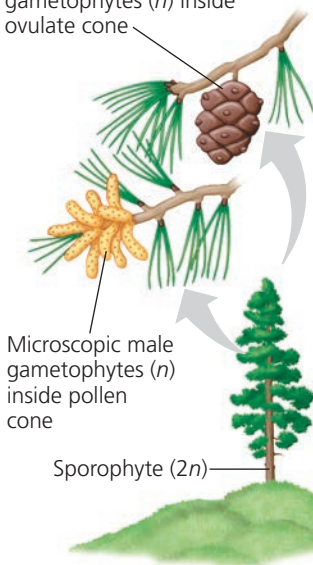
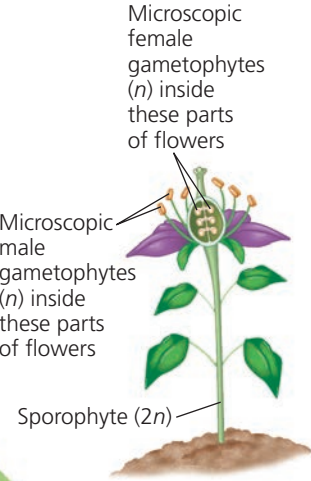
Advantages of Reduced Gametophytes

Mosses and other bryophytes have life cycles dominated by gametophytes, whereas ferns and other seedless vascular

plants have sporophyte-dominated life cycles. The evolutionary trend of gametophyte reduction continued further in the vascular plant lineage that led to seed plants. While the gametophytes of seedless vascular plants are visible to the naked eye, the gametophytes of most seed plants are microscopic.

This miniaturization allowed for an important evolutionary innovation in seed plants: Their tiny gametophytes can develop from spores retained within the sporangia of the parental sporophyte. This arrangement can protect the gametophytes from environmental stresses. For example, the moist reproductive tissues of the sporophyte shield the gametophytes from UV radiation and protect them from drying out. This relationship also enables the developing gametophytes to obtain nutrients from the parental sporophyte. In contrast, the free-living gametophytes of seedless vascular plants must fend for themselves. **Figure 30.2** provides an overview of the gametophyte-sporophyte relationships in nonvascular plants, seedless vascular plants, and seed plants.

Figure 30.2 Gametophyte-sporophyte relationships in different plant groups.

	PLANT GROUP		
	Mosses and other nonvascular plants	Ferns and other seedless vascular plants	Seed plants (gymnosperms and angiosperms)
Gametophyte	Dominant	Reduced, independent (photosynthetic and free-living)	Reduced (usually microscopic), dependent on surrounding sporophyte tissue for nutrition
Sporophyte	Reduced, dependent on gametophyte for nutrition	Dominant	Dominant
Example			Gymnosperm  Angiosperm 

MAKE CONNECTIONS In seed plants, how might retaining the gametophyte within the sporophyte affect embryo fitness? (See Concepts 17.5, 23.1, and 23.4 to review mutagens, mutations, and fitness.)

Heterospory: The Rule Among Seed Plants

You read in Concept 29.3 that most seedless plants are *homosporous*—they produce one kind of spore, which usually gives rise to a bisexual gametophyte. Ferns and other close relatives of seed plants are *homosporous*, suggesting that seed plants had homosporous ancestors. At some point, seed plants or their ancestors became *heterosporous*, producing two kinds of spores: Megaspangia on modified leaves called megasporophylls produce *megaspores* that give rise to female gametophytes, and microsporangia on modified leaves called microsporophylls produce *microspores* that give rise to male gametophytes. Each megasporangium has one megaspore, whereas each microsporangium has many microspores.

As noted previously, the miniaturization of seed plant gametophytes probably contributed to the great success of this clade. Next, we'll look at the development of the female gametophyte within an ovule and the development of the male gametophyte in a pollen grain. Then we'll follow the transformation of a fertilized ovule into a seed.

Ovules and Production of Eggs

Although a few species of seedless plants are heterosporous, seed plants are unique in retaining the megasporangium within the parent sporophyte. A layer of sporophyte tissue called **integument** envelops and protects the megasporangium. Gymnosperm megasporangia are surrounded by one integument, whereas those in angiosperms usually have two integuments. The whole structure—megasporangium, megaspore, and their integument(s)—is called an **ovule** (Figure 30.3a). Inside each ovule (from the Latin *ovulum*, little egg), a female gametophyte develops from a megaspore and produces one or more eggs.

Pollen and Production of Sperm

A microspore develops into a **pollen grain** that consists of a male gametophyte enclosed within the pollen wall. (The wall's outer layer is made of molecules secreted by sporophyte cells, so we refer to the male gametophyte as being *in* the pollen grain, not *equivalent* to the pollen grain.) Sporopollenin in the pollen wall protects the pollen grain as it is transported by wind or by hitchhiking on an animal. The transfer of pollen to the part of a seed plant that contains the ovules is called **pollination**. If a pollen grain germinates (begins growing), it gives rise to a pollen tube that discharges sperm into the female gametophyte within the ovule, as shown in Figure 30.3b.

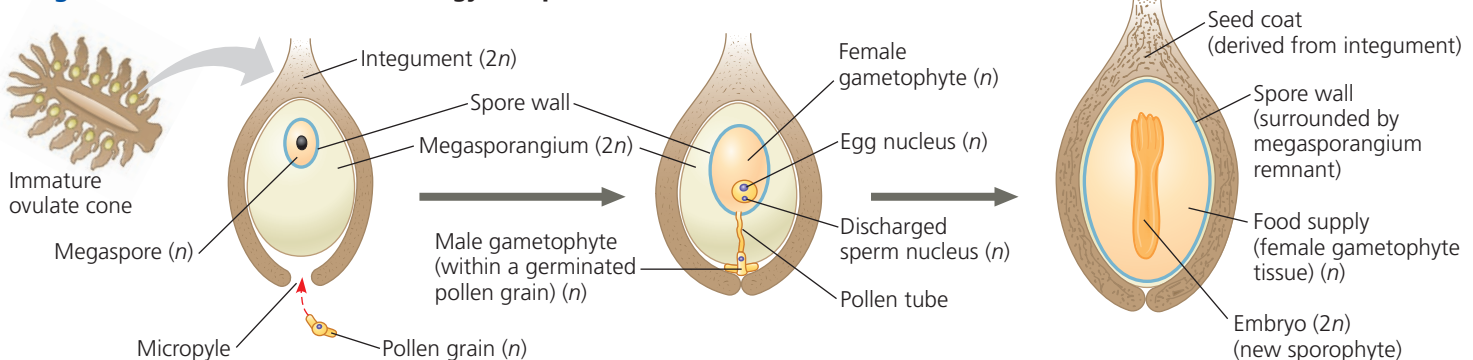
In nonvascular plants and seedless vascular plants such as ferns, free-living gametophytes release flagellated sperm that swim through a film of water to reach eggs. Given this requirement, it is not surprising that many of these species live in moist habitats. But a pollen grain can be carried by wind or animals, eliminating the dependence on water for sperm transport. The ability of seed plants to transfer sperm without water likely contributed to their colonization of dry habitats. The sperm of seed plants also do not require motility because they are carried to the eggs by pollen tubes. The sperm of some gymnosperm species retain the ancient flagellated condition, but flagella have been lost in the sperm of most gymnosperms and all angiosperms.

The Evolutionary Advantage of Seeds

If a sperm fertilizes an egg of a seed plant, the zygote grows into a sporophyte embryo. As shown in Figure 30.3c, the ovule develops into a seed: the embryo, with a food supply, packaged in a protective coat derived from the integument(s).

Until the advent of seeds, the spore was the only protective stage in any plant life cycle. Moss spores, for example, may

▼ **Figure 30.3** From ovule to seed in a gymnosperm.



(a) Unfertilized ovule. In this longitudinal section through the ovule of a pine (a gymnosperm), a fleshy megasporangium is surrounded by a protective layer of tissue called an integument. The micropyle, the only opening through the integument, allows entry of a pollen grain.

(b) Fertilized ovule. A megaspore develops into a female gametophyte, which produces an egg. The pollen grain, which had entered through the micropyle, contains a male gametophyte. The male gametophyte develops a pollen tube that discharges sperm, thereby fertilizing the egg.

(c) Gymnosperm seed. Fertilization initiates the transformation of the ovule into a seed, which consists of a sporophyte embryo, a food supply, and a protective seed coat derived from the integument. The megasporangium dries out and collapses.

VISUAL SKILLS ► Based on this diagram, a gymnosperm seed contains cells from how many different plant generations? Identify the cells and whether each is haploid or diploid.



Figure Walkthrough

SCIENTIFIC SKILLS EXERCISE

Using Natural Logarithms to Interpret Data

How Long Can Seeds Remain Viable in Dormancy?

Environmental conditions can vary greatly over time, and they may not be favorable for germination when seeds are produced. One way that plants cope with such variation is through seed dormancy. Under favorable conditions, seeds of some species can germinate after many years of dormancy.

One unusual opportunity to test how long seeds can remain viable occurred when seeds from date palm trees (*Phoenix dactylifera*) were discovered under the rubble of a 2,000-year-old fortress near the Dead Sea. As you saw in the Chapter 2 Scientific Skills Exercise and Concept 25.2, scientists use radiometric dating to estimate the ages of fossils and other old objects. In this exercise, you will estimate the ages of three of these ancient seeds by using natural logarithms.

How the Experiment Was Done Scientists measured the fraction of carbon-14 that remained in three ancient date palm seeds: two that were not planted and one that was planted and germinated. For the germinated seed, the scientists used a seed coat fragment found clinging to a root of the seedling. (The seedling grew into the plant in the photo.)

Data from the Experiment This table shows the fraction of carbon-14 remaining from the three ancient date palm seeds.

	Fraction of Carbon-14 Remaining
Seed 1 (not planted)	0.7656
Seed 2 (not planted)	0.7752
Seed 3 (germinated)	0.7977

survive even if the local environment becomes too cold, too hot, or too dry for the mosses themselves to live. Their tiny size enables the spores to be dispersed in a dormant state to a new area, where they can germinate and give rise to new moss gametophytes if and when conditions are favorable enough for them to break dormancy. Spores were the main way that mosses, ferns, and other seedless plants spread over Earth for the first 100 million years of plant life on land.

Although mosses and other seedless plants continue to be very successful today, seeds represent a major evolutionary innovation that contributed to the opening of new ways of life for seed plants. What advantages do seeds provide over spores? Spores are usually single-celled, whereas seeds are multicellular, consisting of an embryo protected by a layer of tissue, the seed coat. A seed can remain dormant for days, months, or even years after being released from the parent plant, whereas most spores have shorter lifetimes. Also, unlike spores, seeds have a supply of stored food. Most seeds land close to their parent sporophyte plant, but some are carried long distances (up to hundreds of kilometers) by wind or animals. If conditions are favorable where it lands, the seed can emerge from dormancy and germinate, with its stored food providing

INTERPRET THE DATA

A logarithm is the power to which a base is raised to produce a given number x . For example, if the base is 10 and $x = 100$, the logarithm of 100 equals 2 (because $10^2 = 100$). A natural logarithm ($\ln$) is the logarithm of a number x to the base e , where e is about 2.718. Natural logarithms are useful in calculating rates of some natural processes, such as radioactive decay.

1. The equation $F = e^{-kt}$ describes the fraction F of an original isotope remaining after a period of t years; the exponent is negative because it refers to a *decrease* over time. The constant k provides a measure of how rapidly the original isotope decays. For the decay of carbon-14 to nitrogen-14, $k = 0.00012097$. To find t , rearrange the equation by following these steps: (a) Take the natural logarithm of both sides of the equation: $\ln(F) = \ln(e^{-kt})$. Rewrite the right side of this equation by applying the following rule: $\ln(e^x) = x \ln(e)$. (b) Since $\ln(e) = 1$, simplify the equation. (c) Now solve for t and write the equation in the form " $t = \text{_____}$."
2. Using the equation you developed, the data from the table, and a calculator, estimate the ages of seed 1, seed 2, and seed 3.
3. Why do you think there was more carbon-14 in the germinated seed?



Instructors: A version of this Scientific Skills Exercise can be assigned in MasteringBiology.

Data from S. Sallon et al., Germination, genetics, and growth of an ancient date seed, *Science* 320:1464 (2008).



critical support for growth as the sporophyte embryo emerges as a seedling. As we explore in the **Scientific Skills Exercise**, some seeds have germinated after more than 1,000 years.

CONCEPT CHECK 30.1

1. Contrast how sperm reach the eggs of seedless plants with how sperm reach the eggs of seed plants.
2. What features not present in seedless plants have contributed to the success of seed plants on land?
3. **WHAT IF? >** If a seed could not enter dormancy, how might that affect the embryo's transport or survival?

For suggested answers, see Appendix A.

CONCEPT 30.2

Gymnosperms bear "naked" seeds, typically on cones



Nonvascular plants (bryophytes)
Seedless vascular plants
Gymnosperms
Angiosperms

Extant seed plants form two sister clades: gymnosperms and angiosperms. Recall that gymnosperms have "naked" seeds exposed on sporophylls that usually form cones.

(Angiosperm seeds are enclosed in chambers that mature into fruits.) Most gymnosperms are cone-bearing plants called **conifers**, such as pines, firs, and redwoods.

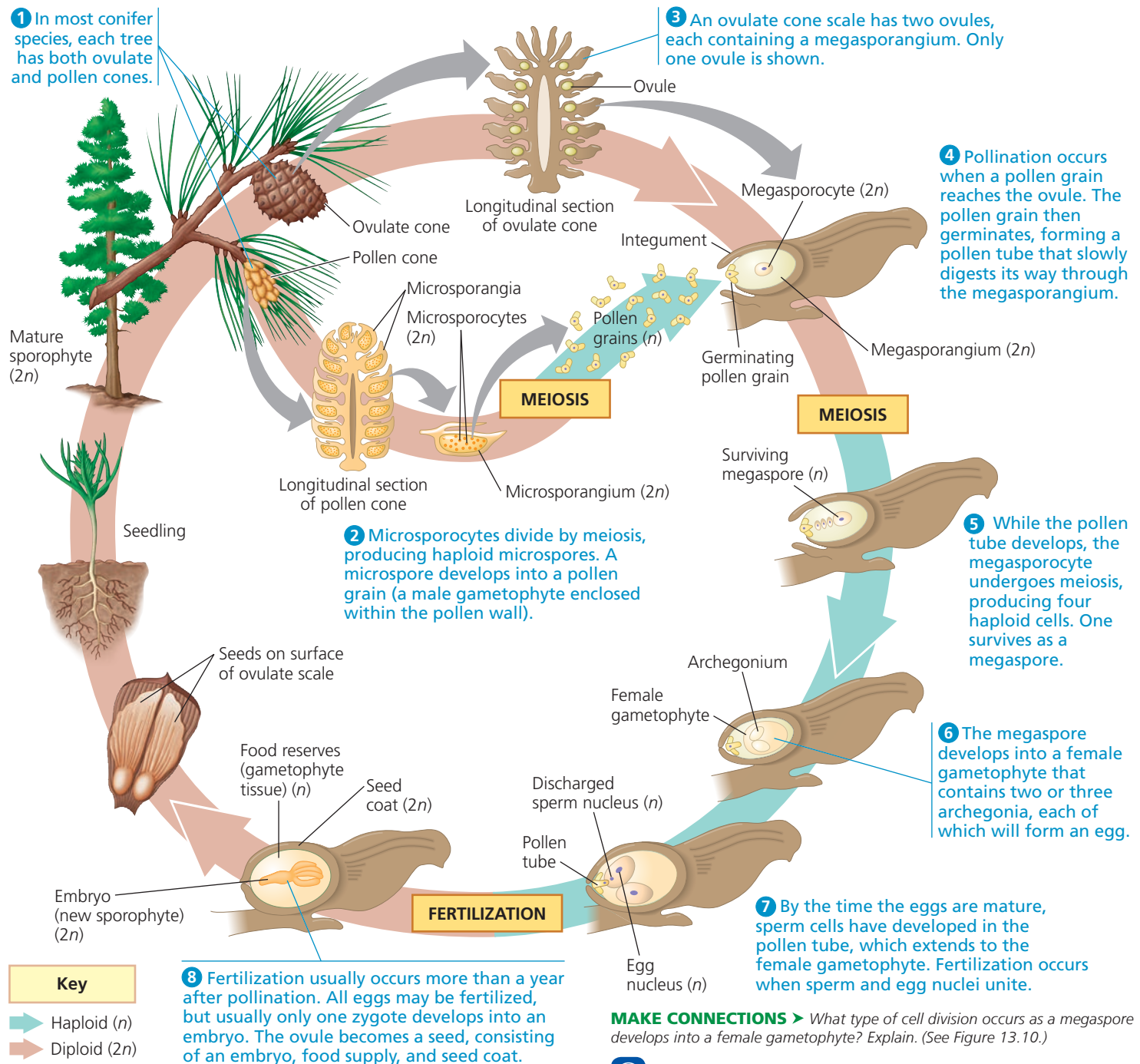
The Life Cycle of a Pine

As you read earlier, seed plant evolution has included three key reproductive adaptations: the miniaturization of their gametophytes; the advent of the seed as a resistant, dispersible stage in the life cycle; and the appearance of pollen as an air-borne agent that brings gametes together. **Figure 30.4** shows

how these adaptations come into play during the life cycle of a pine, a familiar conifer.

The pine tree is the sporophyte; its sporangia are located on scalelike structures packed densely in cones. Like all seed plants, conifers are heterosporous. As such, they have two types of sporangia that produce two types of spores: microsporangia that produce microspores, and megasporangia that produce megaspores. In conifers, the two types of spores are produced by separate cones: small pollen cones and large ovulate cones.

▼ **Figure 30.4** The life cycle of a pine.



Pollen cones have a relatively simple structure: Their scales are modified leaves (microsporophylls) that bear microsporangia. Within each microsporangium, cells called microsporocytes undergo meiosis, producing haploid microspores. Each microspore develops into a pollen grain containing a male gametophyte. In conifers, the yellow pollen is released in large amounts and carried by the wind, dusting everything in its path.

Ovulate cones are more complex: their scales are compound structures composed of both modified leaves (megasporeophylls bearing megasporangia) and modified stem tissue. Within each megasporangium, megasporocytes undergo meiosis and produce haploid megaspores inside the ovule. Surviving megaspores develop into female gametophytes, which are retained within the sporangia.

In most pine species, each tree has both types of cones. From the time pollen and ovulate cones appear on the tree, it takes nearly three years for the male and female gametophytes to be produced and brought together and for mature seeds to form from fertilized ovules. The scales of each ovulate cone then separate, and seeds are dispersed by the wind. A seed that lands in a suitable environment germinates, its embryo emerging as a pine seedling.

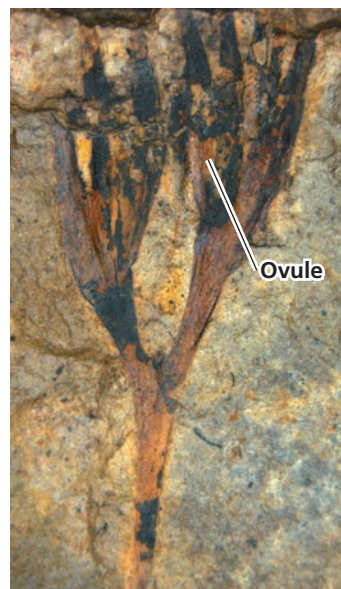
Early Seed Plants and the Rise of Gymnosperms

The origins of characteristics found in pines and other living seed plants date back to the late Devonian period (380 million years ago). Fossils from that time reveal that some plants had acquired features that are also present in seed plants, such as megaspores and microspores. For example, *Archaeopteris* was a heterosporous tree with a woody stem. But it did not bear seeds and therefore is not classified as a seed plant. Growing up to 20 m tall, it had fernlike leaves.

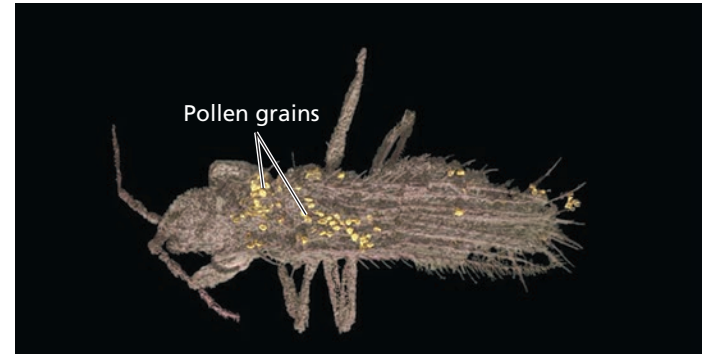
The earliest evidence of seed plants comes from 360-million-year-old fossils of plants in the genus *Elkinsia* (Figure 30.5). These and other early seed plants lived 55 million years before the first fossils classified as gymnosperms and more than 200 million years before the first fossils of angiosperms. These early seed plants became extinct, and we don't know which extinct lineage gave rise to the gymnosperms.

The oldest fossils of species from an extant lineage of gymnosperms are 305 million years old. These early gymnosperms lived in moist Carboniferous ecosystems that were dominated by lycophytes, horsetails, ferns, and other seedless vascular plants. As the Carboniferous period gave way to the Permian (299 to 252 million years ago), the

▼ **Figure 30.5** A fossil of the early seed plant *Elkinsia*.



▼ **Figure 30.6** An ancient pollinator. This 110-million-year-old fossil shows pollen on an insect, the thrip *Gymnopollisthrrips minor*. Structural features of the pollen suggest that it was produced by gymnosperms (most likely by species related to extant ginkgos or cycads). Although most gymnosperms today are wind-pollinated, many cycads are insect-pollinated.



climate became much drier. As a result, the lycophytes, horsetails, and ferns that dominated Carboniferous swamps were largely replaced by gymnosperms, which were better suited to the drier climate.

Gymnosperms thrived as the climate dried, in part because they have the key terrestrial adaptations found in all seed plants, such as seeds and pollen. In addition, some gymnosperms were particularly well suited to arid conditions because of the thick cuticles and relatively small surface areas of their needle-shaped leaves.

Gymnosperms dominated terrestrial ecosystems throughout much of the Mesozoic era, which lasted from 252 to 66 million years ago. In addition to serving as the food supply for giant herbivorous dinosaurs, these gymnosperms were involved in many other interactions with animals.

Recent fossil discoveries, for example, show that some gymnosperms were pollinated by insects more than 100 million years ago—the earliest evidence of insect pollination in any plant group (Figure 30.6). Late in the Mesozoic, angiosperms began to replace gymnosperms in some ecosystems.

Gymnosperm Diversity

Although angiosperms now dominate most terrestrial ecosystems, gymnosperms remain an important part of Earth's flora. For example, vast regions in northern latitudes are covered by forests of conifers (see Figure 51.12).

Of the ten plant phyla (see Table 29.1), four are gymnosperms: Cycadophyta, Ginkgophyta, Gnetophyta, and Coniferophyta. It is uncertain how the four phyla of gymnosperms are related to each other. Figure 30.7 surveys the diversity of extant gymnosperms.

▼ Figure 30.7 Exploring Gymnosperm Diversity

Phylum Cycadophyta

The 300 species of living cycads have large cones and palmlike leaves (true palm species are angiosperms). Unlike most seed plants, cycads have flagellated sperm, indicating their descent from seedless vascular plants that had motile sperm. Cycads thrived during the Mesozoic era, known as the age of cycads as well as the age of dinosaurs. Today, however, cycads are the most endangered of all plant groups: 75% of their species are threatened by habitat destruction and other human actions.



Encephalartos woodii

Phylum Ginkgophyta



Ginkgo biloba



Ginkgo biloba is the only surviving species of this phylum; like cycads, ginkgos have flagellated sperm. Also known as the maidenhair tree, *Ginkgo biloba* has deciduous fanlike leaves that turn gold in autumn. It is a popular ornamental tree in cities because it tolerates air pollution well. Landscapers often plant only pollen-producing trees because the fleshy seeds smell rancid as they decay.

Phylum Gnetophyta

Phylum Gnetophyta includes plants in three genera: *Gnetum*, *Ephedra*, and *Welwitschia*. Some species are tropical, whereas others live in deserts. Although very different in appearance, the genera are grouped together based on molecular data.

► **Welwitschia.** This genus consists of one species, *Welwitschia mirabilis*, a plant that can live for thousands of years and is found only in the deserts of southwestern Africa. Its straplike leaves are among the largest leaves known.



Ovulate cones



► **Ephedra.** This genus includes about 40 species that inhabit arid regions worldwide. These desert shrubs, commonly called "Mormon tea", produce the compound ephedrine, which is used medicinally as a decongestant.



Gnetum gnemon leaves

◄ **Gnetum.** This genus includes about 35 species of tropical trees, shrubs, and vines, mainly native to Africa and Asia. Their leaves look similar to those of flowering plants, and their seeds look somewhat like fruits.



Phylum Coniferophyta

Phylum Coniferophyta, the largest gymnosperm phyla, consists of about 600 species of conifers (from the Latin *conus*, cone, and *ferre*, to carry), including many large trees. Most species have woody cones, but a few have fleshy cones. Some, such as pines, have needle-like leaves. Others, such as redwoods, have scale-like leaves. Some species dominate vast northern forests, whereas others are native to the Southern Hemisphere.

Most conifers are evergreens; they retain their leaves throughout the year. Even during winter, a limited amount of photosynthesis occurs on sunny days. When spring comes, conifers already have fully developed leaves that can take advantage of the sunnier, warmer days. Some conifers, such as the dawn redwood, tamarack, and larch, are deciduous trees that lose leaves each autumn.

► **Douglas fir.** This evergreen tree (*Pseudotsuga menziesii*) provides more timber than any other North American tree species. Some uses include house framing, plywood, pulpwood for paper, railroad ties, and boxes and crates.



► **Common juniper.** The “berries” of the common juniper (*Juniperus communis*) are actually ovule-producing cones consisting of fleshy sporophylls.



◄ **European larch.** The needle-like leaves of this deciduous conifer (*Larix decidua*) turn yellow before they are shed in autumn. Native to the mountains of central Europe, including Switzerland’s Matterhorn, depicted here, this species is extremely cold-tolerant, able to survive winter temperatures that plunge to -50°C .



◄ **Wollemi pine.** Survivors of a conifer group once known only from fossils, living *Wollemi* pines (*Wollemia nobilis*) were discovered in 1994 in a national park near Sydney, Australia. At that time, the species consisted of 40 known trees. As a result of conservation efforts, it is now widely propagated. The inset photo compares the leaves of this “living fossil” with actual fossils.

► **Sequoia.** This giant sequoia (*Sequoiadendron giganteum*) in California’s Sequoia National Park weighs about 2,500 metric tons, equivalent to about 24 blue whales (the largest animals) or 40,000 people. The giant sequoia is one of the largest living organisms and also among the most ancient, with some individuals estimated to be between 1,800 and 2,700 years old. Their cousins, the coast redwoods (*Sequoia sempervirens*), grow to heights of more than 110 m (taller than the Statue of Liberty) and are found only in a narrow coastal strip of northern California and southern Oregon.



► **Bristlecone pine.** This species (*Pinus longaeva*), which is found in the White Mountains of California, includes some of the oldest living organisms, reaching ages of more than 4,600 years. One tree (not shown here) is called Methuselah because it may be the world’s oldest living tree. To protect the tree, scientists keep its location a secret.



CONCEPT CHECK 30.2

1. Use examples from Figure 30.7 to describe how various gymnosperms are similar yet distinctive.
2. Explain how pollen cones differ from ovulate cones.
3. **MAKE CONNECTIONS** > Early seed plants in genus *Elkinsia* are a sister group to a clade consisting of gymnosperms and angiosperms. Draw a phylogenetic tree of seed plants that shows *Elkinsia*, gymnosperms, and angiosperms; date the branch points on this tree using fossil evidence. (See Figure 22.5.)

For suggested answers, see Appendix A.

CONCEPT 30.3

The reproductive adaptations of angiosperms include flowers and fruits



Nonvascular plants (bryophytes)
Seedless vascular plants
Gymnosperms
Angiosperms

Commonly known as flowering plants, angiosperms are seed plants with the reproductive

structures called flowers and fruits. The name *angiosperm* (from the Greek *angion*, container) refers to seeds contained in fruits. Angiosperms are the most diverse and widespread of all plants, with more than 250,000 species (about 90% of all plant species).

Characteristics of Angiosperms

All angiosperms are classified in a single phylum, Anthophyta. Before considering the evolution of angiosperms, we will examine two of their key adaptations—flowers and fruits—and the roles of these structures in the angiosperm life cycle.

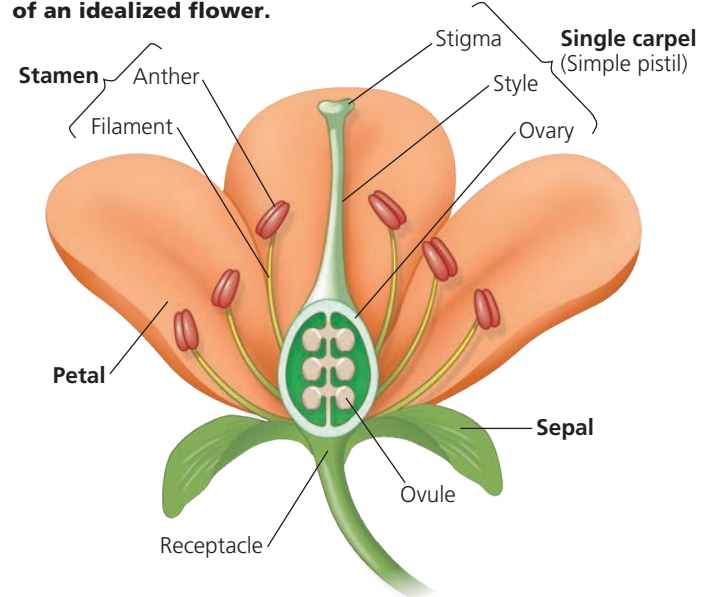
Flowers

The **flower** is a unique angiosperm structure that is specialized for sexual reproduction. In many angiosperm species, insects or other animals transfer pollen from one flower to the sex organs on another flower, which makes pollination more directed than the wind-dependent pollination of most species of gymnosperms. However, some angiosperms *are* wind-pollinated, particularly those species that occur in dense populations, such as grasses and tree species in temperate forests.

A flower is a specialized shoot that can have up to four types of modified leaves called floral organs: sepals, petals, stamens, and carpels (Figure 30.8). Starting at the base of the flower are the **sepals**, which are usually green and enclose the flower before it opens (think of a rosebud). Interior to the sepals are the **petals**, which are brightly colored in most flowers and can aid in attracting pollinators. Flowers that are wind-pollinated, such as grasses, generally lack brightly colored parts. In all angiosperms, the sepals and petals are sterile floral organs, meaning that they do not produce sperm or eggs.

Within the petals are two types of fertile floral organs that produce spores, the stamens and carpels. Stamens and carpels

▼ **Figure 30.8** The structure of an idealized flower.

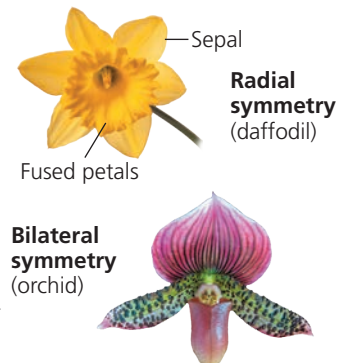


are sporophylls, modified leaves that are specialized for reproduction. **Stamens** are microsporophylls: They produce microspores that develop into pollen grains containing male gametophytes. A stamen consists of a stalk called the **filament** and a terminal sac, the **anther**, where pollen is produced. **Carpels** are megasporophylls: They produce megaspores that give rise to female gametophytes. The carpel is the “container” mentioned earlier in which seeds are enclosed; as such, it is a key structure that distinguishes angiosperms from gymnosperms. At the tip of the carpel is a sticky **stigma** that receives pollen. A **style** leads from the stigma to a structure at the base of the carpel, the **ovary**; the ovary contains one or more ovules. As in gymnosperms, each angiosperm ovule contains a female gametophyte. If fertilized, an ovule develops into a seed.

A flower may have one or more carpels. In many species, multiple carpels are fused into one structure. The term **pistil** is sometimes used to refer to a single carpel (a simple pistil) or two or more fused carpels (a compound pistil). Flowers also vary in symmetry (Figure 30.9) and other aspects of shape, as well as size, color, and odor. Much of this diversity results from adaptation to specific pollinators (see Figures 38.4 and 38.5).

▼ **Figure 30.9** Flower symmetry.

In radial symmetry, the sepals, petals, stamens, and carpels radiate out from a center. Any line through the central axis divides the flower into two equal parts. In bilateral symmetry, the flower can only be divided into two equal parts by a single line.



DRAW IT > Draw the single line that can divide the bilaterally symmetrical flower into two equal parts.

Fruits

As seeds develop from ovules after fertilization, the ovary wall thickens and the ovary matures into a **fruit**. A pea pod is an example of a fruit, with seeds (mature ovules, the peas) encased in the ripened ovary (the pod).

Fruits protect seeds and aid in their dispersal. Mature fruits can be either fleshy or dry (**Figure 30.10**). Tomatoes, plums, and grapes are examples of fleshy fruits, in which the wall (pericarp) of the ovary becomes soft during ripening. Dry fruits include beans, nuts, and grains. Some dry fruits split open at maturity to release seeds, whereas others remain closed. The dry, wind-dispersed fruits of grasses, harvested while on the plant, are major staple foods for humans. The cereal grains of maize, rice, wheat, and other grasses, though easily mistaken for seeds, are each actually a fruit with a dry outer covering (the former wall of the ovary) that adheres to the seed coat of the seed within.

▼ **Figure 30.10** Some variations in fruit structure.

▼ Tomato, a fleshy fruit with soft outer and inner layers of pericarp (fruit wall)



▼ Ruby grapefruit, a fleshy fruit with a firm outer layer and soft inner layer of pericarp



▼ Nectarine, a fleshy fruit with a soft outer layer and hard inner layer (pit) of pericarp



▼ Hazelnut, a dry fruit that remains closed at maturity



▼ Milkweed, a dry fruit that splits open at maturity



As shown in **Figure 30.11**, various adaptations of fruits and seeds help to disperse seeds (see also Figure 38.12). The seeds of some flowering plants, such as dandelions and maples, are contained within fruits that function like parachutes or propellers, adaptations that enhance dispersal by wind. Some fruits, such as coconuts, are adapted to dispersal by water. And the seeds of many angiosperms are carried by animals. Some angiosperms have fruits modified as burrs that cling to animal fur (or the clothes of humans). Others produce edible fruits, which are usually nutritious, sweet tasting, and vividly colored, advertising their ripeness. When an animal eats the fruit, it digests the fruit's fleshy part, but the tough seeds usually pass unharmed through the animal's

▼ **Figure 30.11** Fruit adaptations that enhance seed dispersal.



▶ Some plants have mechanisms that disperse seeds by explosive action.

▶ Wings enable maple fruits to be carried by the wind.



▶ Seeds within berries and other edible fruits are often dispersed in animal feces.

▶ The barbs of cockleburs facilitate seed dispersal by allowing the fruits to "hitchhike" on animals.



 **Animation: Fruit Structure and Seed Dispersal**

digestive tract. When the animal defecates, it may deposit the seeds, along with a supply of natural fertilizer, many kilometers from where the fruit was eaten.

The Angiosperm Life Cycle

You can follow a typical angiosperm life cycle in **Figure 30.12**. The flower of the sporophyte produces microspores that form male gametophytes and megaspores that form female gametophytes. The male gametophytes are in the pollen grains, which develop within microsporangia in the anthers. Each male gametophyte has two haploid cells: a *generative cell* that

divides, forming two sperm, and a *tube cell* that produces a pollen tube. Each ovule, which develops in the ovary, contains a female gametophyte, also known as an **embryo sac**. The embryo sac consists of only a few cells, one of which is the egg.

After its release from the anther, the pollen is carried to the sticky stigma at the tip of a carpel. Although some flowers self-pollinate, most have mechanisms that ensure **cross-pollination**, which in angiosperms is the transfer of pollen from an anther of a flower on one plant to the stigma of a flower on another plant of the same species. Cross-pollination enhances genetic variability. In some species, stamens and

▼ **Figure 30.12** The life cycle of an angiosperm.

